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THE ORGANIZATION OF THE LABORATORIES IN THE MEDICAL CLINIC OF THE JOHNS HOPKINS HOSPITAL.

By Lewellys F. Barker, M. D.,

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Ever since the opening of the Johns Hopkins Hospital the importance of the work of the clinical laboratories has been emphasized. The personal interest and enthusiasm of Dr. Osler in the clinical laboratory work led the members of the medical staff from the beginning to value laboratory investigation as an indispensable accompaniment of the work in the wards. At first the laboratory work consisted in routine examinations and special investigations carried out in small clinical laboratories under the medical wards. For some of the clinical problems attacked these small laboratories were soon found to be insufficient, and in the early years of the clinic, thanks to the courtesy of Dr. Welch, some of the more difficult clinical laboratory investigations were carried on in the pathological laboratory of the university and hospital.

After the medical school was opened the urgent need of facilities for the instruction of medical students in the methods of the clinical laboratory, methods the growing importance of which all recognized, soon became obvious, and special clinical

laboratories were built near the wards for the purpose, the idea being to give every third and fourth year student in the medical school his own laboratory place and outfit for microscopical, chemical, and physical work on materials derived from patients in the wards. In the third year each student is given a systematic training in the use of modern clinical laboratory methods, and in the fourth year the student uses the methods he has learned for the actual investigation of the individual cases assigned to him in his practice as clinical clerk.

This clinical laboratory for instruction, first under the charge of Dr. Thayer, and later under the guidance of Drs. Futcher, McCrae, Emerson, and Boggs successively, has undoubtedly been one of the most important agencies in the education of the students of the Johns Hopkins Medical School; it has been possible as a result of its development to carry on the work at the bedside on a much higher plane than would have been possible without it.

It was soon found that, valuable as such routine clinical laboratory work is for diagnosis and therapy, and contributory as it is to the advance of knowledge by the statistical method, it must be supplemented by original experimental research in order that new methods may be devised and in order that the ever new problems presented by the patients to the clinicians may be solved. From the very beginning of the work of the medical clinic at the hospital the recognition of this investigative, as contrasted with the routine function of the clinical laboratory was appreciated, and the physicians in charge of the laboratory and others associated with them, with the sympathy of Dr. Osler, and largely at his direct suggestion, engaged from time to time in original inquiries which led to results of importance, many of which are recorded in papers in the *Bulletin* and *Reports* of the Hospital.

The physician in charge of the clinical laboratory resided in the hospital and was closely associated with the other members of the resident staff. A clinical bacteriologist also lived in the hospital, directed the bacteriological work of the wards, and engaged in special clinical bacteriological researches. The clinical laboratory thus won for itself a solid place among the clinical disciplines in the Medical School, and made itself a necessary and integral part of the hospital organization.

During the past two years the clinical laboratory has been still further extended, especially on the side of research. In this extension, internal medicine has been influenced by the example of other departments of applied science. Technology has demonstrated how necessary it is for the promotion of the industrial arts to secure investigators trained in the so-called "pure sciences" of physics, chemistry, and biology who will devote themselves to the application of the methods and principles of these sciences to the solution of the special problems by which those who wish to advance the industrial arts are confronted. One has only to recall the tremendous advances in metallurgy, in brewing, in electrical engineering, in the manufacture of arms and ammunition, in sugar refining, and in food preservation to realize how fundamentally significant the interesting of men trained in pure science in the solution of the so-called "practical problems" has been for the extension of knowledge and practice in more strictly utilitarian domains.

Since von Ziemssen recognized the need for similar applications of the fundamental sciences to the solution of the special problems of diagnosis and therapy, and founded clinical research laboratories at the hospital in Munich, the idea has spread, until to-day the work of research by internists in laboratories adjacent to their wards is regarded as essential at least in university clinics.[1] It has occurred to me that a brief description of the organization of the general and special laboratories in the department of medicine at the Johns Hopkins Hospital might be interesting to those who have developed similar laboratories elsewhere and especially to workers in internal medicine contemplating such a development.

The laboratories consist of a general clinical laboratory for instruction and research, laboratories for the routine work of the wards, special laboratories for the application of biological, physiological, and chemical methods to the solution of clinical problems and certain other laboratory facilities to be mentioned further on.

The General Clinical Laboratory for the Instruction and Research and the Routine Ward Laboratories.

(Dr. Thomas R. Boggs in Charge.)

This laboratory, the nucleus of all the clinical laboratories and about which the others cluster, is situated on the second and third floors of the dispensary building and is in direct connection with the biological and clinical divisions and with the wards and outpatient department.

The equipment comprises two large general laboratories for the accommodation of the students where each member of the third and fourth year classes has a locker and desk space. There are in addition, a stock room for supplies or reagents and apparatus of all kinds used in the varied instructive, routine and special work. A general preparation room with Kjeldahl installation and a dark room for spectroscopic and polariscopic work and three rooms for special workers are included.

The functions of the laboratory are two-fold: (a) to give the students a thorough training in the application of all laboratory methods to the diagnosis and investigation of disease. The course extends over the entire academic year and the immediate connection of the laboratory with the wards and the out-patient department makes available an unusually large and varied material. Incidentally this use of the ward material is of no small value to the clinic in diagnosis. For with the simultaneous examination of material by a large number of observers a diagnosis is not infrequently reached which had been overlooked in the more limited examination possible in the wards. (b) The second function of the laboratory is the encouragement of investigation. We are able to offer to special workers laboratory facilities and clinical ma-

[1] Cf. Welch (W. H.). The Evolution of Modern Scientific Laboratories. Johns Hopkins Hos. Bull., Balto., 1896, vii, 19-24.

terial for study along varied lines of medical interest, especially for the study of groups of cases with regard to questions of diagnosis and the results of therapeutic measures.

The development, criticism, and practical application of new methods may here be undertaken to advantage. In addition to this we are able to afford students on the wards special opportunity for detailed study of cases under their charge.

The Routine Laboratory of the wards is also under the direct charge of this department. It has just been entirely remodeled and comprises three laboratory rooms and a stock room. These laboratory rooms are all finished in white tile wainscot and polished cement floors; and have slate-topped tables. Room A is the laboratory for qualitative urinary studies where the routine examination of the specimens from the wards supervised by the resident physician, Dr. Emerson, is conducted by the house officers and the fourth year students. It is equipped with microscopes, centrifuge, and reagents necessary for all ordinary tests.

Room B is devoted to the quantitative work of the routine of gastric analysis, of the determination of sugar, chlorides, and other quantitative analyses ordinarily applied to the ward cases. Special lockers are provided equipped for the different procedures indicated, and the student has the sole use of one of these while he is studying a given case.

Room C is for the study of sputum only, and here all examinations from the hospital and out-patient department are made. No other work is permitted here. Only necessary tables, sinks, and staining appliances are permitted in the room, which is also equipped with a large high-pressure autoclave for the sterilization of the vessels, plates, etc. Adequate provision for the cleansing and disinfection of the hands is also made.

This laboratory for routine work is a natural and valuable feeder to the department of instruction and research in the General Laboratory and the students are encouraged to carry out any investigations requiring more special appliances in the latter.

THE BIOLOGICAL LABORATORY OF THE MEDICAL CLINIC.

(DR. RUFUS I. COLE in Charge.)

Three rooms on the second floor of the surgical building are at present used as a laboratory. A large corner room with windows on two sides offers accommodation for about eight workers. A smaller room adjoining is used by Dr. Cole and one research worker, while a third room is used by the assistant in charge of the routine bacteriological work of the medical department of the hospital. A vault which adjoins these rooms, and which was intended as a receptacle for the medical histories, but which is not needed at present for this purpose, is used as a centrifuge room, as it is found that the noise and vibration is disturbing when the centrifuge is in the same room with workers. The equipment includes an ordinary small electric centrifuge, and also a larger one which revolves eight 25 cc. tubes, with a speed of 3000 revolutions per minute. Such a large centrifuge is necessary in precipitating bacteria from suspensions. In the main room of the laboratory are two large thermostats for the use of workers, and this room is also equipped with lockers and media-closets. Sterilizers and all the apparatus necessary for ordinary bacteriological and biological investigation are kept on hand, and special apparatus required by workers for carrying out their investigations are supplied.

A small animal house for holding a limited number of smaller animals, with yards in which they can be given exercise in the open air, has been built on the hospital grounds. A house for larger animals is needed and will doubtless be supplied as the work progresses. In all experimental work the importance of having the animals kept under good hygienic conditions cannot be over-emphasized.

The purpose of the laboratory is to investigate biological methods of diagnosis and treatment. The possibilities of refinements of diagnosis which the more recent studies of immunity have afforded, make it necessary that these methods should be investigated with the object of rendering them of practical application in the clinic. Such studies also, even if not of immediate practical application, are of great value in adding to our knowledge of the pathogenesis of acute infections. Knowledge of this kind is necessary before we can have any rational specific treatment in the great group of infectious diseases; the discovery of new facts along these lines brings the attainment of this end nearer. There is, therefore, the best of reasons for the establishment of biological laboratories in direct connection with medical clinics. While the problems investigated may at times seem far removed from immediate application to diagnostic and therapeutic purposes, yet it is only by careful investigation of the problems, in immediate contact with a medical clinic, that the applicability of new discoveries can be brought home to the minds of clinical men. In this work there is always a marked tendency to make immediate practical application of results; it should be the purpose of the clinical laboratories to see, first, that methods so applied are based upon sound scientific principles, and secondly, by controlling their actual use upon patients, to determine their real value. The importance, therefore, of men who have been trained not only in special science but also in clinical observation undertaking the solution of such problems is obvious. At present the investigation of methods of specific treatment is left too largely in the hands of manufacturers, whereas it should be the function of the research laboratories of departments of internal medicine. Considerable expense is involved in investigations of the kind mentioned, and it will of necessity be some time before there will be many well-endowed laboratories which can undertake extensive experiments along these lines.

During the past year, on account of the great interest which was stimulated in the opsonic theory and method of treatment by vaccines by the lectures and work of Wright, a considerable part of the work of the biological laboratory in this clinic has dealt with an investigation of problems bearing on this theory.

The biological division of the laboratory is open to all those who are fitted to do experimental biological work, and who are

interested especially in problems pertaining to internal medicine. This year a practical course in immunity was offered to ten students. These men performed the more fundamental immunity experiments; later certain of them undertook the solution of special problems, work upon which is still in progress.

The laboratory offers facilities to members of the medical staff who desire to undertake especial researches, either in connection with patients or upon animals. For such work (especially for those spending much time at the bedside) a thoroughly organized laboratory with easy facilities is indispensable. The laboratory attempts to make its methods and materials easily accessible to qualified inquirers. Post-graduate students working in medicine who desire to spend all or a part of their time in clinical biological reasearch will have problems suggested to them and will be aided in solving them. While the futility of physicians with little preparation (or of those who can devote but a short time to work) undertaking work on such problems is fully realized, those who have adequate preparation, the necessary leisure and a real desire to assist in the discovery of new knowledge are given opportunities and are encouraged.

The special advantages which the laboratory offers to students and physicians working upon the problems of infection and immunity are: (1) Its close association with the medical wards of the hospital, so that clinical material is always available, and (2) the intimate association of its work with that of the medical staff as a whole so that the clinical bearing of new scientific truths is quickly recognized. The division of the laboratory, though only recently established, has already made itself an important part of the medical clinic.

The Physiological Laboratory of the Medical Clinic.

(Dr. Arthur D. Hirschfelder in Charge.)

The physiological division of the clinical laboratory was organized in October, 1905, for the purpose of studying both individual cases and diseased conditions from the standpoint of disturbance of function. It is provided with two small laboratories: One in the New Surgical Building of the Hospital for the clinical study of patients by the special methods in use in physiological laboratories, particularly the graphic methods; the other work-room is in the Hunterian Laboratory of the Medical School. The latter is well equipped for animal experiment, and there the diseased conditions seen in the wards may be reproduced in animals and studied by physiological methods. As far as is possible this is done with a view to establishing by animal experiment the procedures of a true physiological therapy. It has been the aim in this laboratory to bring the studies upon the patient as closely as possible into relation with the findings upon animals and upon mechanical models and to turn these results to practical use. Our experience has fully demonstrated that the best results of animal experimentation are obtained when they are subjected at once to the test of comparison with the conditions found in the patient.

Investigations have been carried on thus far upon some of the problems of secretion by the kidney in experimental nephritis, upon the graphic study of the venous pulse, upon numerous disturbances of cardiac function, paroxysmal tachycardia, Adams-Stokes Disease, and particularly upon the conditions in aortic insufficiency which determine the form of the pulse wave, the variations in maximal and minimal blood-pressure and the failure of the heart. It is hoped in the future to extend the scope of the investigations to the disturbances of secretion and to the study of the pathological physiology of the nervous system, making use of the methods of modern experimental surgery in reproducing, studying, and remedying the conditions.

Optional courses upon the physiological aspects of the diseases of the circulatory system have been offered for students and post-graduates; the disturbances of function are shown as far as possible (1) upon the patient and (2) upon the animal with the structures exposed. Whenever possible the two demonstrations are made upon the same day, otherwise they are made upon successive days; for it is thought that a far better understanding of the clinical condition can be obtained when the experimental picture is fresh in the mind. Further, many minor and even major problems arise at the bedside, which can by laboratory investigation receive elucidation not otherwise possible. It is hoped that in the future the students may be more and more encouraged to look upon their ward and dispensary patients from the physiological standpoint and to investigate the problems arising in their personal experience by the experimental methods.

The Biochemical Laboratory of the Medical Clinic.

(Dr. C. Voegtlin in Charge.)

It is well recognized at the present time that chemistry plays an important rôle in modern medicine. It should be, therefore, the aim of every large hospital and medical school to promote by its aid the more accurate diagnosis and the better treatment of disease. These purposes, however, are by no means easily accomplished because of the relatively elaborate needs that such an undertaking requires. Chemical work is largely dependent upon a laboratory that is furnished with the varied apparatus and materials used in this science. Further, to be of full value to the medical clinic of a hospital, a chemical laboratory must be situated within the walls of the institution, with easy access to the wards, the chemist in charge must have been thoroughly trained in the pure science and he and the members of the hospital staff should be always in close touch. With these ideals in view, and with the support of the hospital and university and of a generous donor who desires that his name shall not be mentioned the biochemical division of the laboratory of the medical clinic of the Johns Hopkins Hospital has been equipped with accommodation for eight workers. Since the establishment of this laboratory last year the work has consisted in investigations bearing upon problems of metabolism, and especially upon the regulation of diet in various diseases, *e. g.*, typhoid fever, nervous diseases, diabetes, and gout. The influence of certain drugs upon the organism, a subject which offers still a large

field for scientific experimental work, is being taken up. The metabolism is studied in cases in which a clinical diagnosis is uncertain or where there are signs of disturbed assimilation of the food or pathological changes in the excretion of the metabolic end-products of the body. Besides this, parts of various organs, that may have been removed from the body as well as blood, exudates and other fluid can be analyzed with the aim of assisting the diagnosis and therapy of special cases.

The value of this kind of work is two-fold. First, it is of benefit to the patients; second, physicians and advanced students are afforded the opportunity of becoming familiar with the general principles of metabolism and nutrition, a subject that is of the utmost importance and one which has hitherto been too much neglected by the medical profession at large.

The chemical division of the clinical laboratory is well equipped for its especial work. The general outfit, though in miniature, is modelled upon the arrangements in the first chemical institute in Berlin, as many conveniences and time-saving devices as possible having been introduced. The facilities for hydrolysis, vacuum distillation, ether recovery, evaporation, combustion, calorimetric work, nitrogen determinations, etc., are very satisfactory. The laboratory is supplied with a liberal variety of Kahlbaum and other pure chemicals, and efforts are always made to provide the special apparatus and supplies which a particular investigation may require.

The work of the past year has led to publications of importance and it is hoped and believed that the inauguration of this division of the clinical laboratory marks an important step forward in the ever-increasing division of labor and specialization which the expansion of the modern medical clinic necessitates.

OTHER LABORATORY FACILITIES IN THE MEDICAL CLINIC.

Though the description of the laboratory facilities of the medical clinic here given deals with the clinical laboratories proper, it would be wrong to allow the opportunity to pass without reference to the continuous advantages to the clinic since the opening of the hospital of the close cooperation between the pathological laboratory of the university and hospital and the wards. The pathological laboratory has been and is still the fountain-head of inspiration for our whole medical organization. Aside from the all-important control of the clinical work by thorough post-mortem examinations the laboratory by its histological, bacteriological, and parasitological studies in connection with the wards, performed, in the earlier days of the hospital, the functions of a true clinical laboratory, and by its more recent expansion at the Hunterian Laboratory in the field of experimental pathological physiology, and experimental surgery it promises to be of even greater service than ever to the allied clinic. A notable innovation, and one which has already proven of great advantage both to staff and students, has been the conduct by Professors Thayer and MacCallum of a clinical pathological conference on Monday afternoons, during which the histories, and intra vitam diagnosis of the clinic are compared (and, when necessary, contrasted) with the findings of the autopsy table.

The laboratories of anatomy, physiology, and pharmacology have also each contributed directly to the work of the medical clinic. A course in clinical medical anatomy is given in the anatomical laboratory, members of the physiological staff have conducted investigations in the wards on blood pressure, on heart block, and on Cheyne-Stokes breathing, and the physiological chemists and pharmacologists have on request helped the workers in the medical clinic in spectroscopic and other special examinations. Without the hearty cooperation and willing self-sacrifice of the various workers in these laboratories devoted to the fundamental medical sciences much that is being accomplished in the work of the medical wards and laboratories would be impossible.

The X-ray laboratory, in charge of Dr. F. H. Baetjer, though primarily a part of the surgical organization of the hospital, has been of very great service to the medical clinic also. In the various forms of arthritis, in diseases of the bones and spine, in gastric and œsophageal disease radiography has been most helpful in diagnosis. Fluoroscopic examinations are now a part of the regular routine in all obscure intrathoracic conditions and the diagnosis of aortic aneurism is now made so easy that there is some danger of neglect of the older methods of arriving at a positive diagnosis of the condition.

For the therapy of the medical clinic the X-ray laboratory has also been drawn into service (skin lesions, exophthalmic goitre, unresolved pneumonia).

The electrodiagnostic and electrotherapeutic laboratory of the clinic form a part of the equipment in the department of neurology of which Dr. H. M. Thomas has charge.

Two additional laboratories are much needed in the medical clinic, one for the study of tuberculosis in the Phipps Dispensary in charge of Dr. Louis V. Hamman, and the other a psychopathological laboratory for the investigation of the psychic side of the cases in the medical wards.

Rooms for the laboratory for the study of tuberculosis are already provided in the Phipps Dispensary and a further sum of money for laboratory purposes has just been donated by the generous giver to whom the medical clinic of the hospital owes this important foundation. This sum, together with the gifts of other friends of the movement to study tuberculosis here, will make it possible to develop the much-needed laboratory side of our tuberculosis work.

At present no funds are available for a psychopathological laboratory. Rooms for the purpose and a laboratory leader trained in the modern methods of the psychological laboratory and especially one versed in the art of psycho-analysis are urgently required, especially pending the endowment and development of that institution so urgently needed here in Baltimore, a university psychiatric clinic.

Enough has been said to make clear the rapid expansion of the clinical laboratory and the benefits to be derived by the clinic from its further growth and work. The papers pub-

lished in this number of the BULLETIN, though representing only a part of the original investigation carried on during the past year, are sufficient to illustrate the character, aims, methods, and results of the laboratory inquiries. The work is still hampered by lack of funds. The time has come when a building of considerable size is needed to house the clinical laboratory and its various subdivisions in order that they may meet the pressing demands that are being made upon them. An endowment which would permit the erection of such a building, equip it, supply salaries for leaders trained in the special sciences so that they can devote their whole time to the work, and meet the annual supply and expense budget would require the setting aside of a large amount of money. Here, surely, is a tempting opportunity for some liberal-minded man or woman who desires to benefit mankind by increasing diagnostic precision and enhancing therapeutic skill.

NOTE ON THE OCCURRENCE OF HOWELL'S NUCLEAR PARTICLES IN EXPERIMENTAL ANÆMIA OF THE RABBIT AND IN HUMAN BLOOD.[1]

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(*From the Laboratory of the Medical Clinic.*)

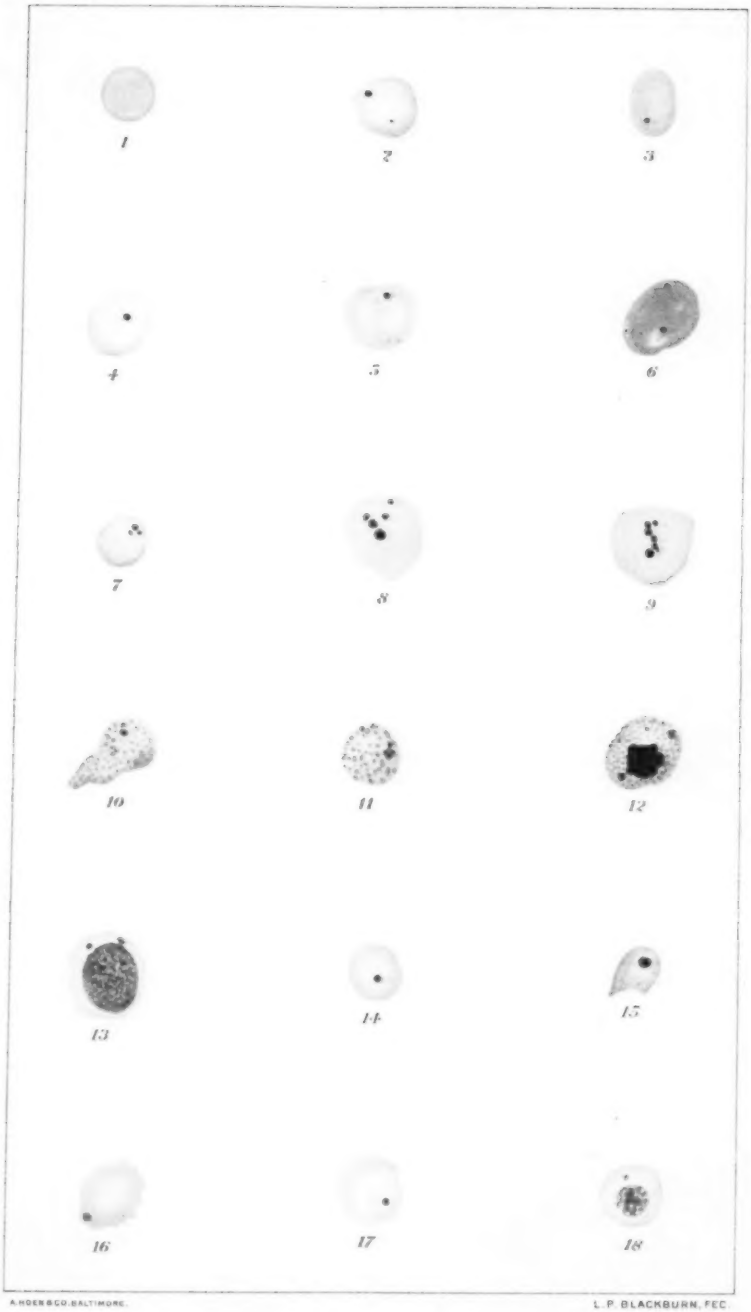
In 1890 Howell (1) discovered in the blood of cats, following a severe hæmorrhage, that a majority of the red blood corpuscles may contain "a single good-sized piece of nuclear matter, too large to be called a granule, but having the shape and appearance of a large nucleolus." This fragment stained readily with methyl green, like the nucleus; it could be seen also in the unstained corpuscles as a refractive particle. It was noted that "the fragment of nuclear matter always lay imbedded in the periphery of the spherical corpuscle." The fragments could not be detached from the corpuscles by shaking or by the addition of water, acetic acid, or other reagents which dissolve out the hæmoglobin of the cell. Moreover, the fragments were not found in the blood of cats normally, but usually followed a severe hæmorrhage. "The only satisfactory explanation of the phenomenon which has occurred to me is that the fragment is a bit of the nucleus left adhering to the corpuscle at the time that the nucleus escaped. Under the conditions necessary for the appearance of the phenomenon, we may suppose that the process of production of new blood cells was vastly accelerated, and that therefore the extrusion of the nucleus was not as perfect as under normal conditions." (Howell.)

Schmauch (2) was the second to study these bodies in the blood of the cat. He called them "endoglobuläre Körperchen." They were found in large numbers (80 per cent of the corpuscles) after venesection and in poisoning with pyrodin and with extract of bothriocephalus latus. In the fresh blood the nuclear particle was seen to vary much in size, from a scarcely visible granule, through all stages up to the size of a nucleus, and to be either round or irregular in shape; some of them showed motility in the fresh blood, usually those which were irregular in form. Fixation of the specimen caused the "endoglobular particles" to shrink, and they were usually round or oval, or possessed of one or two projecting processes. No specific stain could be found for these bodies, but they were stained with all nuclear dyes. The red blood cells which contained the "endoglobuläre Körperchen" presented no abnormalities in form, size, or hæmoglobin content. Erythrocytes possessing nuclear particles were found in all of the internal organs, when present in the peripheral circulation; no local increase in their number was found in any organ or tissue. In a few animals, bone marrow was removed under anæsthesia; no difference was discoverable between it and the circulating blood in the number of red blood cells having nuclear particles. Schmauch believed, in view of the transitions in size from the fully developed nucleus to the smallest "endoglobuläre Körperchen" and the similarity in staining reactions, that these bodies were nuclear in origin and that the loss of the nucleus in the red blood cell of the cat is accomplished not by a breaking up of the nucleus into granules, but rather by a gradual shrinking.

Pol (3), in a study of the pathological morphology of the erythrocytes of the rabbit after phenylhydrazin poisoning, described in a few of the red blood cells "a single, somewhat excentrically placed, coarse, round body, staining intensely with nuclear dyes," which he identified with that found by Schmauch in cats, and by Schmidt (4) in rabbits during poisoning with phenylhydrazin. Schmidt saw similar bodies in the blood of new-born rats and of adult mice. Jolly (5) has also called attention to their constant presence in the blood of new-born white rats and mice; they disappear from the circulating blood shortly after birth. He has found them in the embryo of the white rat and of the mouse (6), and believes, as does Schmauch, that they originate from atrophy of the nucleus.

In the blood of rabbits poisoned with pyrodin (see article in this number of the BULLETIN) I have also met with these small nuclear particles, first described by Howell, in the red

[1] Presented at the Meeting of the American Association of Pathologists and Bacteriologists, at Washington, May 7, 1907.



1. Normal red blood cell.
2. Rabbit. Polychromatophilic red cell containing nuclear particle.
3-13. Pernicious anæmia: 3-5 red blood cells containing single nuclear particles; 6-9, red blood cells containing multiple nuclear particles; 10, 11, red blood cells containing a nuclear particle and basophilic granules; 12, nucleated red blood cell containing two nuclear particles and basophilic granules; 13, megaloblast containing two nuclear particles.
14, 15. Human embryo. Red blood cells containing nuclear particle.
16-18. Typhoid fever (blood crisis): 16, crenated red blood cell containing nuclear particle; 17, polychromatophilic red blood cell containing a nuclear particle; 18, normoblast containing a nuclear particle.
All stained with Hastings' modification of Romanowski's stain.

blood cells. They are, as a rule, very few in number, though occasionally there is a crisis, as many as six having been found in one field (Leitz oil immersion). They do not occur in the blood of normal adult rabbits, so far as I have been able to observe, but may be seen after anæmia becomes established, usually after the count has fallen to about 3,000,000 red blood cells. Their appearance is coincident with that of basophilic granules in the red blood cells, though the cells possessing basophilic granules greatly outnumber those with nuclear particles. The latter were not observed in the fresh blood because of their paucity, but in stained preparations (Fig. 2) they were almost invariably excentrically placed, round or oval in shape—rarely irregular, single (though as many as three have been found in one cell, their size varying considerably); their outline was very sharply defined and occasionally there was noted a paler zone in the protoplasm of the red blood cell surrounding the nuclear particle. They were found in normally staining red blood cells and in those showing polychromatophilia. One fact which was striking in many preparations was the almost complete absence of the deformity (degenerative change) characteristically produced by phenylhydrazin and its derivatives in those cells which contained either a nuclear particle or basophilic granules; this rule was not without exceptions, but it held good in the great majority of instances. The nuclear particles were found rarely in cells possessing a nucleus and in those possessing basophilic granules. Unlike the basophilic granules, the nuclear particle takes on a purple color like that of the nucleus with Romanowski stains. With Ehrlich's triple stain, it seemed that fewer nuclear particles were visible than with hæmatoxylin or with methylene blue.

In the blood of man, Howell's nuclear particles have not been described, so far as I know. In the blood of a patient (A. H., Gen. No. 58,647), recently admitted to Dr. Barker's service, suffering with pernicious anæmia, large numbers of these bodies have been found (Figs. 3 to 13). They were present during and after a blood crisis, in which large numbers of intermediates and megaloblasts, as well as normoblasts were found in the circulating blood (to be reported later by Dr. Krause). The preparations studied were stained with Hastings' stain (modified Romanowski). The nuclear particles resembled morphologically those seen in the rabbit in every particular; but cells containing two or more nuclear particles were very much more numerous here than in the blood of the rabbit (Figs. 6-9, 12, 13). Again, with Hastings' stain, a few nuclear particles were found beautifully stained a brilliant violet (Figs. 5, 7, 10, 11). The majority of the cells containing nuclear particles were of normal or increased size, though many microcytes were seen to possess them, and in most instances the cells with nuclear particles were polychromatophilic, often markedly so, as shown in the accompanying plate. Not infrequently a nuclear particle, often stained violet, was found in a red blood cell with many bluish granules (Fig. 10, 11, 12); the presence of both in the same cell was rare in rabbit's blood. Nucleated red blood cells frequently contained one or more nuclear particles; in some instances the nuclei were fragmenting (Fig. 12), and a filament connecting one or more of the nuclear particles with the larger mass of the nucleus was visible; but usually the particles appeared entirely separated from the nucleus. It is not only in cells having fragmenting nuclei, however, that nuclear particles may be encountered; they have been found in cells with active, perfectly intact nuclei. One megaloblast was found showing a fine chromatin net-work in the nucleus, with two nuclear particles in the surrounding protoplasm (Fig. 13). The presence of such particles in a cell with intact nucleus is of some importance, in that one of the chief arguments against the nuclear origin of the basophilic granules is their occurrence in cells possessing active nuclei. It by no means proves the nuclear origin of the basophilic granules, but it does lessen the value of the argument mentioned. The occurrence of a nuclear particle and basophilic granules in the same cell, does not necessarily mean that the latter are degenerative changes. Nuclear particles are often found in nucleated red blood cells, and it is conceivable that the larger part of the nucleus in such a cell may break up into fine basophilic granules. In man, the nuclear particles are so frequently found in cells having nuclei that their origin through atrophy of the nucleus, as Schmauch and Jolly consider likely in animals (cat, rat, mouse), can be excluded in at least a part of the instances. That both methods of formation may hold good in man is quite possible, *i. e.*, (1) atrophy of the nucleus, or (2) a body derived from the nucleus.

Having seen nuclear particles in the blood of pernicious anæmia associated with a blood crisis, it seemed likely that they would be found in other conditions in man, in which a large number of nucleated red blood cells appeared in the circulating blood. It was not surprising, therefore, to find them in the blood of the human embryo. They were present in small numbers in two cases examined (Figs. 14, 15); one a fœtus 15 cm. long (mother had acute lymphatic leukæmia), the other a fœtus 15 cm. long (mother a healthy colored woman). Furthermore, similar nuclear particles were found in the circulating blood in a patient (Wm. C., Gen. No. 56,567), suffering with typhoid fever, in whose blood there occurred a remarkable crisis with a large number of nucleated red cells (case to be reported in detail by Dr. Emerson). Finally, Dr. Boggs has recently shown me Howell's nuclear particles in the blood of an infant, aged 7 months, suffering with anæmia pseudo-leukæmia infantum; in the blood there were present about 25,000 nucleated red blood cells per cubic millimeter.

The occurrence of nuclear particles in human blood has been associated with the presence of a large number of nucleated red blood cells, *i. e.*, coincident with increased blood formation, as in animals. These bodies are to be looked upon as a sign of regeneration of the blood, cells containing them probably being younger forms of the red blood cell. The nuclear particles in the nucleated red blood cells resemble closely the "basophilic granules" which occur in some of the

lymphocytes in the circulating blood and suggest the possibility of the nuclear origin of the latter.

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BLOOD FORMATION IN THE LIVER AND SPLEEN IN EXPERIMENTAL ANÆMIA.[1]

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It is a well-established fact that the chief function of the bone marrow in adult life is hæmatopoiesis. In embryonic life the liver and spleen are hæmatopoietic organs, but in extrauterine life the marrow unaided seems to be adequate for the formation of the blood and this function is then lost by the liver and spleen, which differ in a corresponding way histologically at these two stages of life. Shortly after birth there is apparently less need for rapid production of blood corpuscles than in the embryo, and this results, therefore, in the absence of recognizable hæmatopoietic (myeloid) tissues in liver and spleen, while at the same time evidence of extensive proliferation is less marked in the bone marrow. If, however, unusual demands are made upon the bone marrow for one reason or another, as, for example, through the loss of a large quantity of blood from hæmorrhage, signs of increased activity are found; and in certain instances, as in most cases of pernicious anæmia, the alteration of the marrow may be such that it resembles in a remarkable manner that seen in the embryo—a reversion of the marrow to the embryonic type, as Ehrlich pointed out many years ago. Here, to all intents and purposes, the problem of the pathological physiology of blood formation in severe anæmias has remained until the recent publications by Meyer and Heineke (1, 2) of the results of their studies, which prove, they believe, a similar reversion to the embryonic type on the part of the liver and spleen in cases of grave anæmia.

In a careful examination, both clinical and histological, of 11 cases of severe anæmia (1) which came to autopsy (seven cases of pernicious anæmia, two of anæmia following sepsis, one of anæmia associated with cardiac disease, and one of so-called leukanæmia) and, in their second more complete study (2), of an additional two cases of pernicious anæmia diagnosed at autopsy, Meyer and Heineke have found strong evidence of blood formation in the liver and spleen. The organs were studied from smears made from the freshly cut surfaces post-mortem and from histological sections. In the spleen they found alterations in all instances; these consisted in decrease in size of the follicles and collections of mononuclear cells, varying greatly in size and in the relation of nucleus to cell body, within the venous sinuses—the so-called Billroth's veins of the spleen. These cells they identified from a study of the smears and sections, as normoblasts, myelocytes, and mononuclear, non-granular, "lymphocyte-like" cells. Changes in the liver were not so constantly present, but were found in seven cases. They were of two kinds: (a) In three instances they found groups of mononuclear cells collected in the liver capillaries, chiefly in the peripheral portion of the lobules, consisting of lymphocyte-like cells, normoblasts, and myelocytes, and (b) in four cases there were collections of large mononuclear cells rich in protoplasm in the periportal connective tissue, usually arranged about the vessels, with many mononuclear eosinophile cells also. In the smears made from both spleen and liver the proportion of nucleated red blood cells and myelocytes greatly exceeded that found in preparations made from the blood. The liver sections corresponded very closely in appearance with those of the human embryo's liver at about the seventh month, while the spleen was also embryonic in type. No collections of cells like those described in the liver and spleen were found in any other organs, and the authors felt justified in the belief that the changes represented true blood formation rather than a wandering in of cells from the circulation, the cell-nests resembling closely those seen in the bone marrow.

With the view of determining whether similar changes could be reproduced in animals by the administration of substances known to cause anæmia, experiments were begun by the writer at the suggestion of Prof. Müller and Dr. Meyer. The rabbit was the animal chosen and pyrodin (acetylphenyl-

[1] Presented before the American Association of Pathologists and Bacteriologists at Washington, D. C., May 7, 1907.

[2] These experiments, begun in Prof. Müller's laboratory, have been continued in the laboratory of the medical clinic of Prof. Dock of the University of Michigan and in that of Prof. Barker of Johns Hopkins University.

hydrazin) was selected as the toxic agent. The attempt was made to produce a chronic anæmia of rather severe grade, so that the bone marrow would be overtaxed in blood formation. By this means it was hoped that the liver and spleen would reassume one of their embryonic functions and assist in the production of blood.

The animals were kept in well-cleaned and ventilated cages and were fed with oats and greens daily. Frequent examinations of the blood were made, including counts of both red and white cells, estimation of hæmoglobin, and smears. Pyrodin was given about 5 p. m., the dose depending upon the blood count made earlier in the afternoon. A solution of pyrodin in water was prepared in which 1 cc. equaled 0.005 gram pyrodin.

Experiment A. Rabbit I. Male. Weight, 2665 grams. Pyrodin administered by stomach tube.

Date. 1905	R. B. C.	W. B. C.	Hb.		Pyrodin	Remarks
30-VI	5,550,000	12,000				
3-VII	5,200,000	7,080			0.015	
4-VII					0.01	
5-VII	5,350,000	9,600	60%	(Sahli)	0.02	
6-VII	4,090,000				0.02	
7-VII	3,650,000	14,732	43%	"	0.03	
8-VII	2,875,000				0.02	
10-VII	2,105,000	17,166	25%	"	0.02	No free Hb. in serum.
11-VII	2,200,000				0.02	
12-VII	2,155,000	11,776	17%	"	0.02	
13-VII	1,830,000				none	Animal weak. Wt. = 1964 gms.
14-VII	1,940,000	13,954	25%	"	"	
15-VII	2,375,000	11,354	31%	"	"	
17-VII	2,435,000	5,820	46%	"	0.02	
18-VII	3,490,000	5,154	46%	"	0.03	Fresh solution of pyrodin.
19-VII	3,125,000				0.035	Serum clear.
20-VII	2,635,000	8,660	37%	"	0.03	
21-VII	2,265,000				0.03	Serum faintly pink.
22-VII	1,910,000	15,466	26%	"	0.03	Abscess of left ear.
24-VII	1,080,000	18,688	25%	"	none	
25-VII	1,930,000				0.03	
26-VII	1,937,000	9,622	34%	"	0.03	
27-VII	2,245,000				0.04	
28-VII	1,785,000	12,622	31%	"	0.03	Fresh solution of pyrodin.
29-VII					0.02	
30-VII	1,875,000	11,266	29%	"	0.03	
31-VII	1,230,000				0.02	
1-VIII	1,450,000	33,822	23%	"	0.025	Ear abscess evacuated.
2-VIII	1,925,000				0.03	
3-VIII	1,565,000	8,600	23%	"	0.03	
4-VIII	1,385,000					Died.

Death occurred some time between 11.45 a. m. and 2.45 p. m. At autopsy the body was warm. There was slight rigor mortis in the legs. Weight = 2065 grams. All of the organs were very dark in color. The spleen was enlarged, measuring 8 x 1.5 x 2 cm. The bone marrow was dark reddish brown in both femurs. The lungs were air-containing throughout and there were numerous pin-head hæmorrhages on the surfaces. The kidneys showed pigmentation of the cortex; this was especially marked along the inner edge. Heart muscle was paler than normal. There was very little blood in the organs. No fluid in peritoneum, pleuræ, or pericardium.

MICROSCOPICAL EXAMINATION.—The tissues, fixed in alcohol, mercuric chloride and acetic acid, and formaldehyde, were embedded in paraffin and in celloidin, sectioned, and stained with hæmatoxylin and eosin, van Gieson's stain, and borax carmine with potassium ferrocyanide and hydrochloric acid.

Bone marrow.—The marrow of the shaft of the femur, normally fatty in full-grown rabbits, showed marked hyperplasia, the fatty marrow being entirely replaced by myeloid tissue. A study of smears, stained with hæmatoxylin and eosin, May-Grünwald's stain, and Ehrlich's triple stain, in conjunction with that of the sections, shows that the non-granular mononuclear cells (myeloblasts) are greatly in the majority. There are many normoblasts and many intermediates, but no typical megaloblasts. Myelocytes are also numerous. There are a few phagocytes and many megalokaryocytes (see Fig. 1).

Spleen.—There is marked destruction of the cells of the pulp. The follicles are diminished in size and in some instances the central artery is almost completely devoid of lymphoid tissue. *In the venous sinuses* there are large *nests of mononuclear cells* with non-granular protoplasm and rather deeply staining nuclei (see Fig. 2). There is an enormous number of phagocytes containing fragments of red blood cells and brown amorphous pigment, both in the capillaries and venous sinuses and in the pulp. Megalokaryocytes are fairly numerous. *Smears* made from the freshly cut surface of the spleen show many non-granular mononuclear cells like those in the bone marrow, varying in size from that of a red blood cell to a cell whose diameter is twice as large or greater, the protoplasm being very basophilic, the nucleus a little paler (May-Grünwald stain). Many of the nuclei have brick red blotches which extend at times into the protoplasm. From the former cell one sees apparently all gradations to the typical myelocyte, *i. e.*, from cells having few pseudo-eosinophile or eosinophile granules to those having many. There is a moderately large number of myelocytes. In the smears there are also a few normoblasts. Very few polymorphonuclear pseudoeosinophiles are seen and rarely one finds a mastcell. There are many pale, free nuclei. Phagocytes are present.

Liver.—The section of the liver presents a remarkable picture. The liver cells are well preserved, but show considerable pigmentation, hæmosiderin being present in large amount. The *liver capillaries* are much *widened, and in them, especially in the periphery of the lobules* (see Fig. 3), are *nests of mononuclear cells* (Fig. 4), having non-granular protoplasm and deeply staining nuclei (hæmatoxylin and eosin). The cells of the intracapillary nests resemble large lymphocytes, though at times apparent erythroblasts (normoblasts) and

undoubted pseudoeosinophile myelocytes are seen. Some of the cells show mitotic figures. The number of cells seen in the central veins of the lobules and in the larger vessels of the liver is relatively much less than that in the capillaries of the peripheral part of the lobules, while in the central zone of the lobules nucleated cells are very scarce in the capillaries. Occasionally a *megalokaryocyte* is found, often in connection with a collection of mononuclear cells (see Fig. 5), at times independent of a cell-nest. In the capillaries there are also phagocytes like those seen in the spleen. No collections of mononuclear cells are found in Glisson's capsule, though there is a myelocyte occasionally. *Smears* made from the liver resemble in every way those made from the spleen.

Kidneys.—These organs show no noteworthy alteration except for marked hæmosiderosis of the cortex.

Lungs.—There is moderate congestion. A few megalokaryocytic emboli are seen.

The remaining organs showed nothing of importance.

In this experiment we have, then, a chronic anæmia produced by administration of pyrodin in which a maximal effort has been made on the part of the organism to regenerate the blood. Not only is the bone marrow hyperplastic, but the liver and spleen which present a very striking resemblance to those organs in the rabbit's embryo during the stage when they are actively engaged in blood formation, may be assumed to aid in the formation of blood. The picture parallels that described by Meyer and Heineke in pernicious anæmia in man to a striking degree in the presence of myeloid tissue in both liver and spleen, and further resemblances to pernicious anæmia are found in the high color index which existed during life, the blood crises, the presence of "nuclear particles" in the red blood cells (to be described in another paper), the myeloblastic type of bone marrow, the phagocytosis in the hæmatopoietic organs, and the hæmosiderosis of liver, spleen, and kidneys.

Experiment B. Rabbit II. Male. Weight, 1285 grams. Pyrodin given subcutaneously and by stomach tube.

Date. 1905	R. B. C.	W. B. C.	Hb.		Pyrodin	Remarks.
1-VII	5,200,000	9,000				
3-VII				Gm.	0.01	
4-VII	3,055,000	7,140	40%	(Sahli)	0.005	
5-VII					0.005	
6-VII	2,700,000	9,732	32%	"	0.005	
7-VII	2,900,000				0.01	
8-VII	2,675,000	14,600	35%	"	0.01	
10-VII	2,350,000				0.01	
11-VII	2,655,000	16,110	41%	"	0.01	Hb. estimation verified.
12-VII	2,265,000				0.01	
13-VII	2,465,000	8,510	35%	"	0.015	Wt. = 1335 gms.
14-VII	2,180,000	10,688			0.01	
15-VII	2,330,000	5,576	37%	"	0.015	
17-VII	2,410,000	11,600	36%	"	0.02	
18-VII	2,610,000				0.025	Fresh solution of pyrodin.
19-VII	2,585,000	10,844	38%	"	0.03	
20-VII	2,160,000				0.02	
21-VII	2,060,000	25,532	32%	"	0.025	
22-VII	1,725,000				0.02	
23-VII	1,212,000	33,488	26%	(Sahli)	none	
24-VII	1,835,000				0.02	
25-VII	2,085,000	10,510	35%	"	0.03	
26-VII	1,262,000				0.02	
27-VII	950,000	16,510	21%	"	none	
28-VII	1,775,000				0.025	
29-VII	1,950,000	26,310	34%	"	0.03	
30-VII	1,850,000				0.03	
31-VII	1,720,000	19,800	22%	"	0.03	
1-VIII	1,480,000				0.035	
2-VIII	1,365,000	21,044	22%	"	0.03	
3-VIII	1,500,000				0.03	
4-VIII	1,375,000	25,954	25%	"	none	
5-VIII	1,475,000				0.03	Pyrodin by stomach tube.
7-VIII	2,100,000	14,354	38%	"	0.03	Pyrodin by stomach tube.
8-VIII	2,230,000				0.04	Pyrodin by stomach tube.
9-VIII	2,125,000	30,250	25%	"	0.04	Fresh solution of pyrodin.
10-VIII	1,415,000				0.04	Pyrodin by stomach tube.

Death during the night. Autopsy at 12.15 p. m. There was a small amount of clear, light yellow fluid in the peritoneum. Right lung showed moderate hypostasis and œdema. Otherwise the organs resembled in every particular those from rabbit I.

Microscopical examination.—The tissues from this rabbit, as well as those from the remaining animals, were all treated as in rabbit I, experiment A.

Bone marrow.—This resembles in all respects that seen in rabbit I. In the smears, however, there are a few nucleated red cells which may be classed as megaloblasts.

Spleen.—There is marked necrosis of the pulp and the Malpighian follicles are reduced in size. In places in the venous sinuses there are groups of mononuclear, non-granular cells like those in experiment A. In one group there were two megalokaryocytes and several phagocytes, and in one of the non-granular cells a mitotic figure was seen. Hæmosiderosis is marked. The erythrocytes throughout the spleen are for the most part shrunken and distorted. *Smears*, stained as in experiment A, show very many cells resembling lymphocytes with a moderate number of normoblasts and myelocytes which, however, seem less numerous than in rabbit I. There are many phagocytes and practically every red blood cell is distorted.

Liver.—There is marked fatty degeneration, affecting chiefly the cells of the central part of the lobule. There is no dilatation of the capillaries and no intracapillary nests of cells are to be seen. No megalokaryocytes are found. There is considerable hæmosiderosis. *Smears* show a few polymorphonuclear pseudoeosinophiles. Very rarely one finds a normoblast or a myelocyte; they seem to be about as numerous as they are in the heart's blood. Mastzellen are comparatively numerous. A few phagocytes are seen.

Kidneys.—Marked hæmosiderosis of the cortex; otherwise practically negative.

The remaining organs are negative.

The spleen, which has been active in this experiment as a hæmatopoietic organ, has assisted the bone marrow in the attempt to compensate for the anæmia, but there is no evidence of hæmatopoiesis in the liver.

Experiment C. Rabbit III. Male. Weight, 2170 grams. Pyrodin given by stomach tube.

Date. 1906	R. B. C.	W. B. C.	Hb.	Pyrodin	Remarks.
11-IV	5,050,000	9,020	53% (Miescher)	none	
18-IV	6,025,000	12,400	62% "	0.01	
19-IV	5,820,000			0.02	
23-IV	3,850,000	12,500	44% "	0.02	Rabbit seems lifeless.
24-IV	3,487,000			0.025	
25-IV	3,275,000	14,160	34% "	0.04	
26-IV	2,125,000			none	
27-IV	1,843,000			"	
28-IV	1,093,000	12,480	23% "	"	
30-IV	1,925,000	7,000	39% "	"	
1-V	3,441,000	8,600	47% "	0.03	
2-V	2,075,000	10,640	39% "	none	
3-V	3,721,000	9,280	51% "	"	Difficulty in passing tube.
4-V	3,833,000	5,600	51% "	"	Difficulty in passing tube.
5-V	4,087,000	6,932	58% "	0.035	
7-V	2,975,000	12,532	42% "	0.03	
8-V	2,750,000	13,120	33% "	0.035	
9-V	2,600,000	15,600	32% "	0.035	
10-V	2,450,000	26,800	25% "	0.04	
11-V	1,500,000	26,300	17% "	none	
12-V	1,400,000	20,400	19% "	0.025	
14-V	2,312,000	5,849	28% "	0.04	
15-V	2,300,000	6,620	26% "	0.04	Fresh solution of pyrodin.
16-V	1,993,000	19,520	23% "	0.04	
17-V	1,858,000	8,700	15% "	none	
18-V	1,730,000	7,464	16% "	"	
19-V	1,935,000	3,776	21% "	0.04	
21-V	2,566,000	4,088	28% "	0.045	
22-V	2,020,000	7,064	20% "	none	
23-V	2,012,000	17,600	22% "	0.04	
24-V	1,762,000	5,552	18% "	none	
25-V	1,525,000	8,900	17% "	"	
26-V	1,735,000	7,464	16% "	"	
28-V	2,225,000	3,700	28% "	0.045	
29-V	2,360,000	5,200	19% "	0.045	
30-V	1,880,000	16,800	15% "	none	
31-V	1,293,000	21,500	12% "	"	
1-VI	1,044,000	18,932	11% "	"	
2-VI	1,341,000	10,044	11% "	"	

Death occurred between 10 and 11 a. m. on June 4, 1906. Autopsy at 3 p. m.

There were small yellowish nodules in the liver, some of which had extended to the surface of the organ; they were slightly elevated, flat across the top, and rather firm in consistency. No areas of softening were found in them on section. In other respects the organs differed, macroscopically, in no essentials from those in the preceding rabbits.

MICROSCOPICAL EXAMINATION.—*Bone marrow.*—There is marked hyperplasia of the myeloid tissue, the granular marrow cells (myelocytes) being in the majority. The islands or cell-nests described by Bunting are well seen and many of the large non-granular cells in the center of the nests show karyokinetic figures. Phagocytes are present. In the *smears* there are many free pseudoeosinophilic granules; there are very few intact myelocytes.

Spleen.—This resembles the spleen in rabbits I and II in the decrease in size of the splenic follicles, the diminished number of cells in the pulp, and the presence of a few megalokaryocytes. Phagocytes are present in enormous numbers. A few pseudoeosinophile myelocytes are found in the pulp. In the venous sinuses and rarely in a capillary, collections of mononuclear cells resembling large lymphocytes are seen; at times a megalokaryocyte is present in these collections of mononuclear cells. Evidences of mitosis are not lacking in the cells collected in the venous sinuses. *Smears* show large numbers of nucleated reds, as many as six being found in one field (Leitz 1/12 oil immersion; ocular IV). The majority of the nucleated reds are normoblasts, though there are many intermediates and rarely a megaloblast (?). No definite cell division figures are found in the smears. There is a great number of lymphocyte-like cells resembling the non-granular, mononuclear cells of the bone marrow. A moderate number of pseudoeosinophile myelocytes is present. Mastzellen are very scarce.

Liver.—The liver cells show little change other than a moderate pigmentation of the cells of the peripheral zone of the lobules. In the liver capillaries, both in the central and peripheral zones, there are many pseudoeosinophile leucocytes, mostly polymorphonuclear with only an occasional mononuclear. Megalokaryocytes are not seen. Glisson's capsule is unaltered. The nodules found at autopsy present a central necrotic area surrounded by granulation tissue. *Smears* show many polymorphonuclear pseudoeosinophiles, very few normoblasts and mastzellen, few pseudoeosinophile myelocytes.

The remaining organs are negative except for pigmentation of the renal cortex.

Experiment D. Rabbit IV. Male. Weight, 2360 grams. Pyrodin given subcutaneously.

Date. 1906	R. B. C.	W. B. C.	Hb.	Pyrodin	Remarks.
12-IV	5,855,000	4,176	55% (Miescher)	none	
18-IV	5,662,000		59% "	0.01	
19-IV	5,860,000	6,800	59% "	0.015	
23-IV	5,612,000		51% "	0.015	
24-IV	4,820,000	5,200	44% "	0.03	
25-IV	5,170,000			0.04	
26-IV	3,125,000	8,844	43% "	0.03	
27-IV	1,664,000			none	
28-IV	1,406,000	6,920	11% "	"	
30-IV	1,440,000	2,800	17% "	"	
1-V	1,941,000	2,664	26% "	"	
2-V	3,360,000	2,488	39% "	0.03	
3-V	3,164,000	3,100	45% "	0.035	
4-V	2,714,000	4,700	43% "	0.03	
5-V	2,400,000	6,400	43% "	0.03	

Date. 1906	R. B. C.	W. B. C.	Hb.	Pyrodin	Remarks.
7–V	2,100,000	7,128	35% (Miescher)	0.03	
8–V	2,125,000	13,800	25% "	0.035	
9–V	1,662,000	18,080	15% "	none	
10–V	1,237,000	4,932	18% "	"	
11–V	1,807,000	5,376	24% "	0.025	
12–V	2,014,000	3,864	28% "	0.035	
14–V	1,468,000	4,480	22% "	0.03	
15–V	1,785,000	4,852	20% "	0.035	
16–V	1,262,000	11,200	15% "	none	
17–V	1,750,000	4,932	19% "	"	
18–V	2,160,000	2,640	24% "	0.035	
19–V	2,050,000	3,100	23% "	0.035	
21–V	1,281,000	3,360	12% "	none	Animal weak. Fur rough.

Animal died between 11 a. m. and 1 p. m. on May 22, 1906. Autopsy at 5 p. m. Large red clots in both ventricles and extending into the aorta; practically no fluid blood in any of the vessels or organs. The latter are macroscopically the same as in rabbit I. As in the preceding animals, no lymph glands or hæmolymph glands were found.

Microscopical examination.—*Bone marrow.*—The section shows marked hyperplasia of the bone marrow of the myeloblastic type. In the *smears* the myeloblasts are by far the most numerous, the erythroblasts and granular marrow cells being relatively few in number.

Spleen.—The alterations in the spleen resemble those seen in the three previous experiments, but they are less marked. Megalokaryocytes are present. *Smears* show, in addition to many lymphocyte-like cells, a few normoblasts and myelocytes.

Liver.—Sections show very little alteration. In the *smear* only two normoblasts were found after prolonged search, not more than smears from the blood showed, it seemed.

Kidneys.—Marked pigmentation of the convoluted tubules is found.

Lungs.—There is marked œdema and moderate congestion. In the capillaries one finds a few megalokaryocytes.

Experiment E. Rabbit V. Female. Weight, 1900 grams. Pyrodin given by stomach tube.

Date. 1906	R. B. C.	W. B. C.	Hb.	Pyrodin	Remarks.
16–X	5,500,000	8,200	61% (Miescher)	0.01	
17–X	5,500,000	8,240	62% "	0.02	
18–X	4,330,000	9,760	57% "	0.02	
19–X	4,310,000	6,240	48% "	0.035	
20–X	3,425,000			none	
22–X	2,055,000			"	
23–X	1,840,000	9,280	29% "	"	
24–X	2,105,000	7,200	39% "	"	
25–X	2,415,000	5,680	39% "	0.025	
26–X	2,880,000	7,760	40% "	0.035	
27–X	2,990,000	6,000	43% "	0.045	
29–X	2,410,000	7,360	34% "	0.045	
31–X	2,265,000	6,320	31% "	0.04	
1–XI	2,735,000	5,680	38% "	0.05	
2–XI	2,600,000	3,680	29% "	0.03	Fresh solution of pyrodin.
3–XI	2,510,000	6,160	26% "	0.04	
5–XI	2,585,000	17,840	29% "	0.04	
6–XI	2,310,000	5,600	30% "	0.04	
7–XI	2,660,000	5,520	35% (Miescher)	0.045	
8–XI	1,850,000	9,000	25% "	none	
9–XI	2,070,000	6,640	30% "	0.04	
10–XI	2,150,000	5,280	30% "	0.045	
12–XI	2,275,000	7,040	30% "	0.05	
13–XI	2,090,000	4,720	26% "	none	
14–XI	2,730,000	8,720	34% "	0.07	
15–XI	2,775,000	5,760	31% "	0.14	

Rabbit was found dead at 8 a. m. Autopsy at 11 a. m. Rigor mortis present. Spleen 8.5 x 1.5 x 0.5 cm. Right lobe of liver greatly atrophied. Two small nodules in liver and the normal mottling of the organ lost. Very small amount of slightly reddish ascitic fluid. Otherwise the findings at section were the same as in rabbit I.

Microscopical examination.—*Bone marrow.*—Both sections and smears show myeloblastic hyperplasia of the bone marrow.

Spleen.—The section resembles closely that from rabbit IV. A few pseudoeosinophile myelocytes are seen in the meshes of the pulp and there are a few megalokaryocytes. There are small nests of mononuclear, non-granular cells, which look like lymphocytes, in the venous sinuses. *Smears* of the spleen show many lymphocyte-like cells, rarely a nucleated red blood cell, and a few myelocytes.

Liver.—There is some cloudy swelling and pigmentation of the liver cells. No nests of cells are to be seen in the capillaries. The smears are negative.

Lungs show moderate œdema. There are no megalokaryocytes in the capillaries.

The other organs, excepting the kidneys which present the usual changes, are negative.

Experiment F. Rabbit VI. Female. Weight, (?)—average size. Pyrodin given by stomach tube.

Date. 1906	R. B. C.	W. B. C.	Hb.	Pyrodin	Remarks.
16–X	4,090,000	8,080	61% (Miescher)	0.01	
17–X	4,705,000	11,640	60% "	0.02	
18–X	4,025,000	8,640	52% "	0.02	
19–X	4,260,000	10,000	52% "	0.035	
20–X	3,590,000				Animal weak.
22–X	1,535,000			none	

At about 10 a. m. on October 23, 1906, rabbit died. Autopsy at 3.30 p. m. The organs were all negative except for marked anæmia. There was very little fluid blood. No bleeding on section of liver. Clotted blood in heart, arteries, and veins. Bone marrow grayish brown. Spleen 5 cm. long.

Microscopical examination.—*Bone marrow.*—The fatty marrow of the femur is largely replaced by cellular myeloid tissue in which there are a few normoblasts and a few myelocytes, the majority of the cells being non-granular mononuclears (myeloblasts). There are many megalokaryocytes and a few phagocytes. *Smears* reveal nothing additional.

Spleen.—The pulp is poor in cells. The capillaries and venous sinuses are greatly widened and filled with blood. The follicles are slightly diminished in size. There is a moderate

number of phagocytes. A few very small collections of mononuclear, non-granular cells are found in the venous sinuses. In the *smears* many cells resembling the myeloblasts of the marrow are present; there are very few myelocytes and no normoblasts seen.

Liver.—There is nothing unusual with the exception of a few giant cells in the liver capillaries. Smears show very few non-granular mononuclear cells like those seen in the spleen; no normoblasts or myelocytes are found.

Kidneys.—Slight cloudy swelling and pigmentation of the cortex.

Lungs.—An occasional megalokaryocytic embolus is to be seen.

No cause can be found for the rapidly progressive pernicious course of the anæmia in this case. The rabbit received exactly the same doses of pyrodin (and on the same days and hours) as rabbit V; in the one the blood count gradually fell till exitus lethalis occurred less than nine days after the beginning of the intoxication; in the other the fall in the number of the erythrocytes was less pronounced and a fatal issue did not result. The probable explanation would seem to be, in part at least, defective powers of hæmatogenesis in rabbit VI, such as one sees in the so-called aplastic pernicious anæmia in man. This assumption is further supported by the practical absence of nucleated red blood cells from the circulating blood during the entire course of the anæmia. In this case there was not, however, aplasia of the blood forming organs. It is true that there was not complete myeloid transformation of the fatty marrow of the femur, and evidence of hæmatopoiesis in the spleen, if present, was slight, but it is uncertain whether the hyperplasia of the blood forming organs in this instance is any less than that which might be found in the rabbit ordinarily after an acute anæmia lasting a litle more than eight days. No alterations, other than those characteristic of pyrodin poisoning, were found in any of the organs, and there is, therefore, a similarity between the result obtained in this experiment and certain cases of "aplastic" anæmia in man, for in the latter the disease process results, it seems probable, from excessive blood destruction with little or no evidence of compensatory blood formation.

The earliest attempt at studying hæmatopoiesis in anæmia of adult animals experimentally was made by Bizzozero and Salvioli (3) in 1881. After venesection in guinea-pigs and dogs they found large numbers of nucleated red blood cells in the spleen, which normally contains few, as well as in the bone marrow. In rabbits, whose spleen contains no nucleated reds normally in adult life, they were unable to produce changes similar to those obtained in guinea-pigs and dogs. They believed, as a result of their experiments, that the spleen was active in regenerating the blood. Later Gibson (4) repeated their experiments on dogs in part, with the same result, and he made the observation that many of the nucleated reds in the spleen presented division figures in the nuclei, a point strongly in favor of their local origin. In 1890 there appeared the work of Howell (5), in which he was able to show that, after severe and repeated bleedings, and in some instances after a single strong hæmorrhage, nucleated red blood corpuscles were demonstrable in the spleen of the cat with every indication that they were multiplying there, though normally these cells are not found in the cat's spleen in postnatal life.

In studying the spinal cord changes occurring in experimental anæmia of rabbits produced by pyrodin, von Voss (6) noted that there was a deposition of granular pigment in the spleen with areas of necrosis, fatty degeneration in the liver, and in the kidneys all stages of parenchymatous nephritis. Tallquist (7) directed his attention especially to the iron content of the organs of dogs, in which both acute and chronic anæmia had been produced by the administration of pyrodin and of pyrogallol, and was able to prove in many instances a marked increase in the iron of the liver with considerable deposition of hæmosiderin in the spleen, kidneys, and bone marrow frequently. In my own experiments there was a marked reaction for hæmosiderin in liver, spleen, and kidneys in rabbits I and II, the only ones in which it was tried, but the equally marked pigmentation of the cells in the remaining animals makes it probable that the same holds true in all six. In the bone marrow in my experiments the pigment is contained chiefly in phagocytes. In only one instance was fatty degeneration of the liver found, as von Voss reported, while in none of my animals were the renal changes sufficiently marked to consider the existence of a nephritis. Very recently Rothmann and Mosse (8) have studied the effect of chronic pyrodin poisoning in dogs and give additional results of the general findings at autopsy (Mosse). No changes were found in the lymph glands. The spleen, enlarged at autopsy as in the reports of all previous workers, contained much pigment and the follicles were entirely preserved; the characteristic pulp cells were not well preserved. The kidneys showed the usual changes, they say, in the epithelial cells of the straight and convoluted tubules. Hæmosiderosis of the liver was noted. Most interesting was the condition of the hyperplastic bone marrow, similar to that described by Reckzeh (9) in dogs after pyrogallol-anæmia. The cells often designated "Stammzellen" or myeloblasts were present in very large numbers, there were many normoblasts and few granular cells. Unlike others, Reckzeh described megaloblasts in addition to normoblasts in the bone marrow. The marrow of the femur in my experiments showed myeloblastic hyperplasia in all instances with the exception of rabbit III, in which there was a chronic infection, a fact which may explain the large numbers of granular cells.

Lastly, and of greatest interest in connection with the present work, Bunting (10) in 1906 showed, among other things, that chronic anæmia of rabbits, produced by the administration of saponin, lead in some instances to collections of cells in the venous sinuses of the spleen, just as Meyer and Heineke had found in man and as I found in my first two experiments.[2] "The peripheral venous sinuses of the spleen were much dilated and crowded with cells of the marrow type

[2] Mentioned in the preliminary report of Meyer and Heineke, 1905. q. v.

chiefly of the erythrogenetic series, but including many megalokaryocytes and leucocytes. The nucleated red blood cells were grouped much as in the marrow and showed numerous mitotic figures. The veins of other organs are practically free from nucleated red cells, except for an occasional small group in the liver and the constant presence of megalokaryocytic nuclei in the capillaries of the lung." In Bunting's rabbits the anæmia did not become very severe, and this he attributed to the vicarious blood formation occurring in the spleen. It seems much more likely, however, that in some way tolerance to the poison was established, for in my first experiment, where evidence exists of hæmatopoiesis in bone marrow, spleen, and liver as well, there developed, nevertheless, a profound anæmia with fatal issue. As in his animals, I have found megalokaryocytes in the capillaries of the lungs, but not constantly. They were not present in the liver capillaries in Bunting's experiments.

The anæmia produced by pyrodin is due, not to any interference with normal blood formation, so far as is known, but to a great increase in blood destruction. Pyrodin acts upon the red blood cells causing shrinkage and deformity and, as Heinz (11) has demonstrated, these effects are most pronounced about 24 hours after the administration of the drug. In my experimental animals the color index remained high, as in Tallquist's experiments. Fortunately in the present work, the complete blood examination was made always between 22 and 24 hours after the administration of the pyrodin. The changes in the red blood cells will be discussed in another paper. Suffice it for present purposes to say that the deformities in the red blood corpuscles which Heinz described occurred in all of my rabbits. The serum was examined several times for the presence of free hæmoglobin, but none was found, an experience similar to Tallquist's, where excessive doses were not employed. Study of the histological sections shows beyond a doubt, it seems, that the injured red blood corpuscles are taken up by phagocytes which are found in very large number in the spleen and in much smaller number in the liver and bone marrow. In a very short time all the injured cells are removed from the circulating blood, unless, possibly, a few recover and are able to functionate. This phagocytosis of red blood cells occurring in the spleen, liver, and bone marrow in experimental animals is of particular interest, since Warthin (12) has demonstrated a like occurrence in the spleen, lymph glands, hæmolymph glands, and bone marrow in pernicious anæmia in man. We have, then, produced experimentally an anæmia which may be, and probably is, like primary pernicious anæmia in its origin; in neither is there hæmoglobinæmia as a rule, though this may exist exceptionally, and in both injured red blood corpuscles are removed from the circulating blood by phagocytes found in the hæmatopoietic organs which possess the double function of forming and "cleaning" the blood.

In embryos Köllicker showed many years ago—and it is now generally accepted—that the liver is the chief and earliest hæmatopoietic organ. Later in fœtal life the spleen also assumes this function and finally the bone marrow becomes effective in blood formation. Toward the end of intrauterine life, and in the early part of post-natal life the liver and spleen cease forming blood, a function which is reserved solely for the bone marrow. As was noted before, Ehrlich has shown the similarity between the bone marrow of the embryo and that of many patients dying of pernicious anæmia. And Meyer and Heineke demonstrated a like analogy between the blood-forming liver and spleen of the embryo and the same organs in pernicious anæmia. They have also called attention recently to a further point of resemblance of embryo's blood with that seen in pernicious anæmia in the existence of a high color index in each and elsewhere I shall bring forward still another analogy in the presence of "Howell's nuclear particles" in the blood of the human embryo and in that of pernicious anæmia in man. It is evident, therefore, that the work of Meyer and Heineke has marked a distinct advance in the pathology of pernicious anæmia, in that they have shown, so far as it is capable of demonstration at present, that there is not a defective regeneration of the blood in pernicious anæmia (excepting aplastic anæmia), but rather a very great increase in blood formation, the spleen and, in some instances, the liver assuming this function.

In the present experiments it is not possible to prove absolutely that the liver and spleen have reverted to their embryonic condition and taken up the function of blood formation, but it is possible to say that they present the histological pictures seen in the liver and spleen of the rabbit's embryo during the stage of intrauterine life when it is believed that these organs are actively engaged in hæmatopoiesis, and the inference is, therefore, perfectly logical that their function is the same here as it is during fœtal life. That evidence of hæmatopoiesis exists in the spleen in practically all of my experiments and in the liver in only one instance may be explained by the fact that the spleen, which is the last to assume its blood-forming power, is the first to regain it, whereas the liver, beginning its hæmatogenetic function at an earlier period of intrauterine life than the spleen, re-assumes it with greater difficulty.

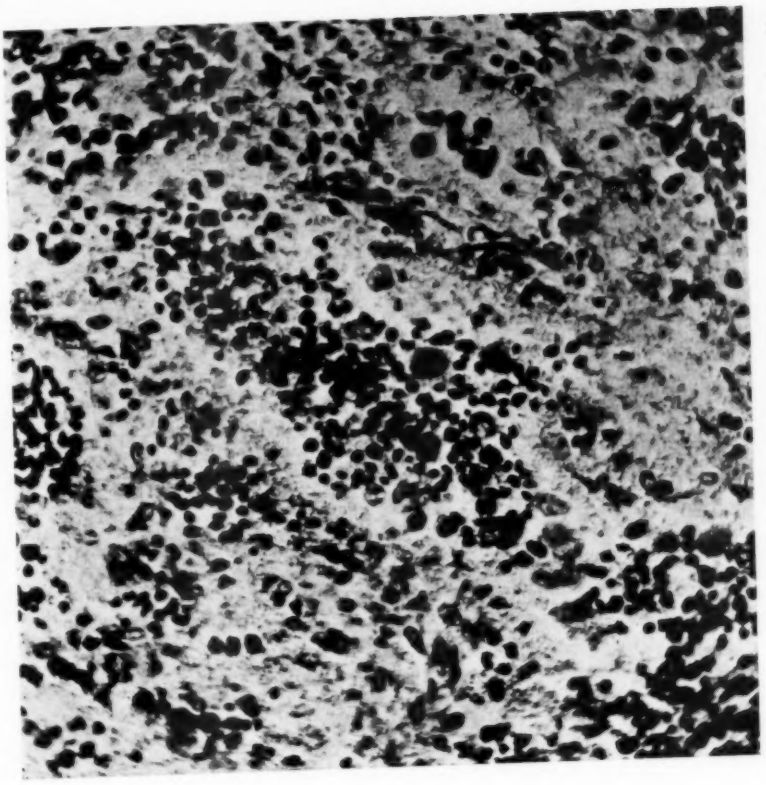
Conclusions.

1. The anæmia produced in rabbits by the administration of pyrodin (by stomach tube or subcutaneously) is one with a high color index and results from injury to certain of the red blood corpuscles which are then removed from the circulating blood by phagocytes in the spleen, bone marrow, and liver. This resembles the condition found in pernicious anæmia in man.

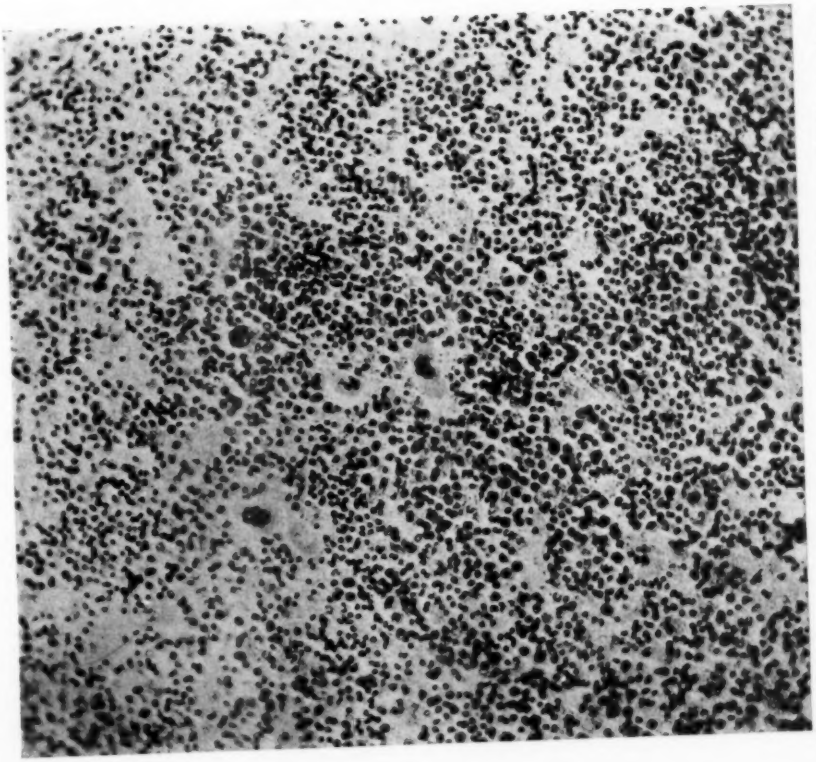
2. The increased blood destruction leads to increased (compensatory) blood formation.

3. The stimulus to increased regeneration of the blood, whatever its nature may be, leads to heightened activity of the hæmatopoietic function of the bone marrow, the occurrence of myeloid elements in the spleen and occasionally in the liver.

4. The changes occurring in the liver and spleen in the experimental animals are similar histologically, so far as the



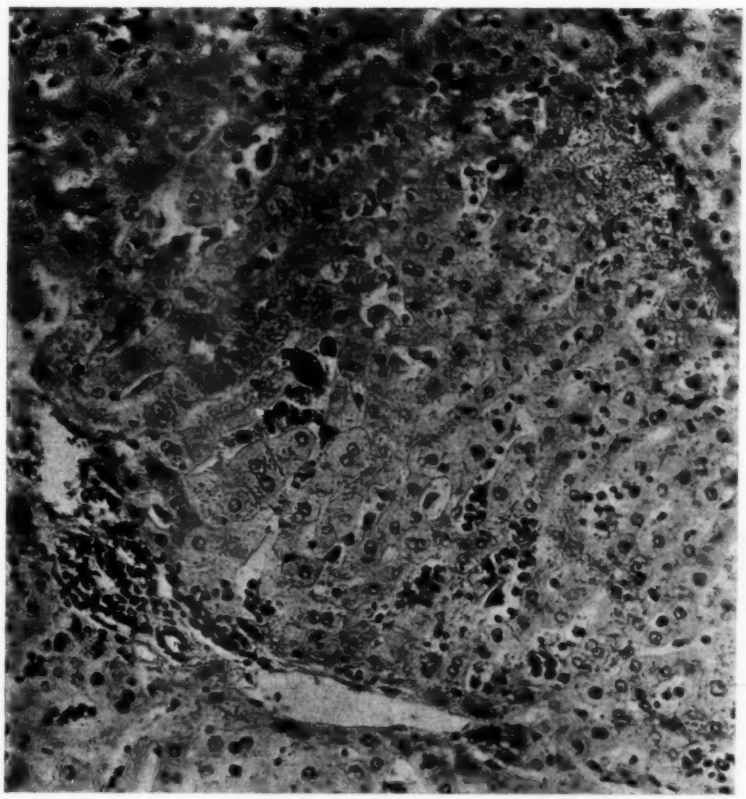
2. Photo-micrograph of the spleen of rabbit I, experiment A, showing (in the center) a venous sinus follicle with mononuclear cells having non-granular protoplasm.



1. Photo-micrograph of the bone marrow of rabbit I, experiment A, showing (myeloblastic) hyperplasia.

4. Photo-micrograph of the liver of rabbit I, experiment A, showing the intracapillary nests of mononuclear cells having non-granular protoplasm. (High magnification.)

3. Photo-micrograph of a liver lobule of Rabbit I, experiment A, showing the arrangement of the intracapillary nests of cells in the peripheral part of the lobule. (Low power.)



5. Photo-micrograph of the liver of rabbit I, experiment A, showing several intracapillary nests of mononuclear cells and, in one of them, a megalokaryocyte. (High magnification.)

hæmatogenetic cells are concerned, to those seen in the normal rabbit's embryo at certain stages in its development, and it may be assumed, therefore, that the spleen and liver have taken up their embryonic function, *i. e.*, hæmatopoiesis.

5. The return of the embryonic function is in the reversed order of its disappearance.

6. Hæmosiderosis of the organs occurs as in pernicious anæmia of man.

7. The weight of experimental evidence favors the theory of increased blood destruction (the toxic theory) rather than that of decreased blood formation as the chief factor in the production of primary pernicious anæmia in man.

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MEASUREMENT OF THE COAGULATION TIME OF THE BLOOD, AND ITS APPLICATION.

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This work was undertaken in 1904, at Dr. Osler's suggestion, to test the value of Milian's new method of determining the coagulation time; and under Dr. Boggs' direction it was gradually broadened to include a comparative study of all the methods and their application.

A synopsis of the paper is as follows:

I. History of development.
II. General remarks.
III. Boggs modification of the Brodie-Russell instrument.
IV. A practical modification of Milian's slide method.
V. Coagulation times in pathological cases and calcium therapy.

SECTION I. HISTORY OF DEVELOPMENT.

A discussion of the development of this subject should naturally follow a chronological order. But this we do not propose to do. The development has been along three different lines, according to three different types of methods employed, as the following schema makes evident; and it shall be the key to our brief description.

I. Hayem's method.
  Slide methods.
    1. Testing with needle.
    2. Microscopical appearance of fibrin shreds.
    3. Watch glasses.
    4. Milian's method.
      Bezançon and Labbé's modification.
    5. Author's modification.
  Platinum loop method.
II. Capillary tubes:
  (a) Vierordt's methods.
  (b) Wright's methods.
III. Brodie and Russell method:
  (a) Pratt-Grützner modification.
  (b) Boggs modification.

Hayem (1) discusses the clinical value of a determina-

tion of the coagulability of blood and the deductions possible. His method consists in filling a flat-bottomed glass test-tube to a constant mark with blood as it flows from the wound. Time is counted from the issue of the blood from the vessels, and coagulation is completed when tipping the tube does not influence the shape of the clot. His results are very discordant. He could get nothing satisfactory, and says so. His times varied so in one and the same animal that he practically abandoned further observations he had planned. However, the method has been used by Dastre and Floresco (2) and by Brat (3), the latter much preferring it to Vierordt's or Wright's methods for accuracy. Brat states that the blood adheres to the test-tube walls in two minutes, and is immobile in three.

Bezançon and Labbé (4) describe Hayem's "éprouvette" more accurately. It is about 5 centimeters high, with an interior diameter of 7 mm., and is filled with blood to a height of 3 to 4 cm., being closed with a cotton tampon. The end-point is as Hayem described, but they make the method less accurate by recording as the beginning point, the moment when the first drop falls into the cylinder, instead of its appearance in the wound. They give 10 to 20 minutes as the normal coagulation time by this method.

The slide methods are classified with Hayem's, because the better of them depend upon the changes in the surface of the blood, or its contour, during movements of the slide, just as Hayem's does in the moving of the test-tube.

The simplest method (5) received the blood upon glass slides, and the consistency was tested from time to time with needles. When fibrin shreds were demonstrated in the gelatinous blood, coagulation had occurred. It is very evident no accurate work can be done with such crude technique.

An improvement (5) upon this was the study under the microscope for the first appearance of fibrin shreds. Schwab (6) recently revived this method. A small drop on a slide under a coverslip will show by the oil immersion a central zone of many layers of red blood cells and a peripheral ring, clear and fairly free from them. In the clear zone, the fibrin shreds are sought, and the time of their first appearance noted. He adds that the end point is often difficult to determine sharply, and the method is full of opportunities for gross errors. 5 to 6 minutes is the normal time.

Vierordt (7) mentions a method of which he made no use, however, simply testing it in the development of the method which now bears his name. It consisted in putting a drop of blood between two watch glasses. At first it is relatively fluid, flowing according to the movements of the glasses. The end-point is noted when the drop adheres to the glass.

In 1904, Milian completed a method of determining and recording the coagulation time of blood in capillary hæmorrhages, first suggested in 1901 (8). The clinical application was presented by René Jacquot (9). Using Hayem's test tube, Milian noted the difference in the coagulability of the blood at the beginning and at the end of a hæmorrhage. He then studied a hæmorrhage in detail. Taking as the beginning point the time the finger was pricked, he let the drops fall upon a series of numbered glass slides, noting the time the first drop was received and recording each successive drop,—also the time the last drop fell. "Coagulation of a drop is completed when one can tip the glass slide vertically without the drop upon it changing its shape, a moment easy to determine. When not coagulated, the drop takes the shape of a tear on moving the slide; but remains convex in outline when coagulated." Thus determined, the coagulation time of the first drop in a hæmorrhage of 41 drops was 17 minutes; of the last (41st), 15 minutes; of the 20th, as typical of the intermediates, 23 minutes 5 seconds. A drop obtained by pressure after the hæmorrhage had ceased, clotted in 6 minutes.

It was found constantly that the first and last drops of a capillary hæmorrhage coagulated more rapidly than the majority of the intermediates, and the last more quickly than the first. This he termed "coagulation dissociée" or "sur lame." For preserving these records, he devised a special chart (10), which is long and tedious, and which he himself soon discarded.

As completed (11), his technique is somewhat different from the original method. The blood is allowed to drop from the finger prick onto the slide, the slide not being touched to the drop as he had advised originally. The observations are to be made in as identical conditions of ventilation, temperature, and surroundings as possible. Coagulation has occurred when a uniform convexity in the contour of the drop is preserved on tilting the slide vertically. When the blood is still fluid, it sags to the lower side.

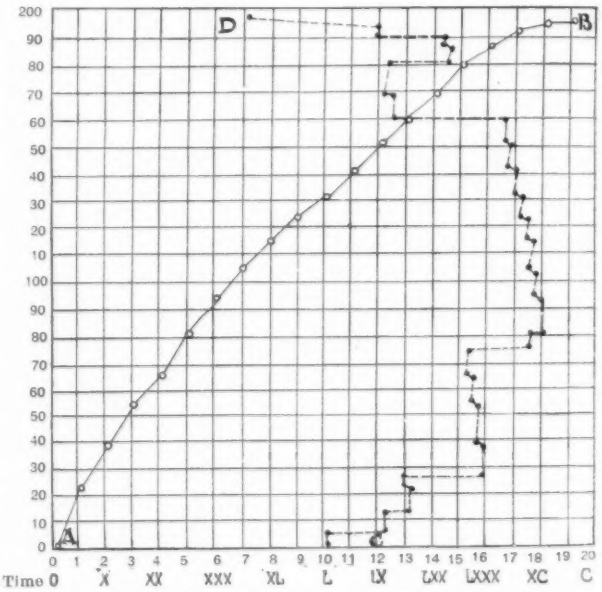

Fig. 1.—Milian's curves of coagulation and hæmorrhage.

The results are recorded graphically upon a chart. The Curve of Hæmorrhage and the Curve of Coagulation are both plotted (see Fig. 1). For the curve of hæmorrhage (*A-B*)

the vertical divisions mark the minutes of the hæmorrhage, the horizontal ones the drops per minute. This shows the speed of flow.

In the curve of coagulation (*C-D*) the vertical divisions are in Roman numerals to indicate duration of coagulation in minutes. Here the curve of hæmorrhage shows 197 drops flowing in 19 minutes, at a rate of 23 for the first minute, 10 for the tenth minute, and 2 for the eighteenth. The curve is typical, approaching the horizontal more and more as the hæmorrhage is ceasing.

The curve of coagulation shows the first drop to clot in 51 minutes, the ninetieth in 90 minutes, and the hundred and ninety-seventh in 35 minutes.

In this method the recording chart has the advantage of giving considerable information about a capillary hæmorrhage in a brief, concise form. This we think a very important point. The more data given, the more inferences can be drawn. As his terms imply, this is a record of much more than the simple coagulation time of the blood. It pictures the powers of the local tissues in their defensive reaction. Of course, the duration of the hæmorrhage depends largely upon the depth of the puncture and the vascularity of the tissues. However Arthus (12), Delezenne (13), and Milian (14) have all shown that the influence of the skin is a vital factor —a most important point to bear in mind.

For comparative laboratory methods, this might be good; but the complexity and labor involved make it impracticable for bedside and ward work. A simpler method is more desirable. In the first place it requires, as a rule, much more than the customary puncture to obtain a hæmorrhage in which the blood will flow with such readiness as Milian's records show. The blood must drop from the finger. It is unusual to be able to get more than five or six drops by spontaneous flow from a wound with the lancet. From our experience, we believe an incision with a scalpel is necessary to obtain such records as 40 to 50 drops. We have found it difficult, even in this way, to obtain as many as 197 drops in what we could conscientiously call a capillary hæmorrhage and Milian (11) himself states that we must confine ourselves to capillary hæmorrhages alone in these practical observations.

The second objection is that there are so many drops which must be tipped up fairly often and their profiles studied, that it is a question if two observers can work fast enough together to catch the exact moment when each one of the drops is coagulated. When the end points begin to appear, it seems as if they all come at once. In one of our cases 28 drops reached their end points inside of 4½ minutes. The first drop to coagulate was the last received. 22 of the 28 drops were found clotted within a period of 2 minutes—so that accurate work was impossible. The limit of error is in direct ratio to the number of drops, and must be considered in a hæmorrhage such as is charted above.

Thirdly, the times obtained are less comparable to the action of blood in a wound than in any other method. It is hard to imagine a drop of blood spread out to about 1 cm. diameter on a glass slide remaining fluid from 60 to 90 minutes, as some of his records show. When a drop is received by letting it fall on the slide, as in this method, it varies from .6 to 1.25 cm. in diameter—about 1 cm. on the average. After coagulation has occurred, there is so much serum under the fibrin that the contour of the drop still changes on tipping the slide vertically. By waiting until this serum evaporates sufficiently to allow the contour to remain convex on moving the slide, one can obtain "coagulation times" of 60 and 90 minutes.

Bezançon and Labbé (4) describe a method evidently based upon Milian's. Three drops are allowed to fall upon three well-cleaned glass slides. Placed under a bell-jar, they are examined as in Milian's method, from time to time to determine the stage of coagulation. The fresh drop is convex upon the slide, but tends to flow towards the lower side in tilting the slide. As coagulation commences, the drop is deformed only slightly in the vertical position, and remains convex and immobile when the process is completed. The normal time by this method is about 10 minutes, 20 to 45 minutes being pathological. It differs from Milian's method in a lack of completeness in describing the characteristics of the hæmorrhage. Single drops cannot be compared when there is no uniformity of size. Furthermore, they make the error of counting time from the moment when the drop falls from the ear,—not when it appears in the wound. No records are quoted in detail.

The author's modification of Milian's method is described in detail in Section IV of this paper. It is practically the same as that recommended by Bezançon and Labbé but those details are carefully attended to which make it a reliable method for comparative results. The beginning point is the moment at which the blood appears in the wound. The end point is determined as by Milian. The drop is not allowed to fall upon the slide, but care is taken to always observe the same sized drop—4 mm. in diameter being adopted. A series of drops are picked up by touching a slide to the drops. The slide is placed upon a millimetre rule and those drops not wanted are wiped off. This can be made comparable to almost any other method by using a larger or smaller drop, according as the other method tends to have a longer or shorter average time. Detailed description and application follows in Section IV.

Mention[1] may be made here of a method in use in the Middle West, in which the blood is picked up in a series of platinum loops, and at intervals of one-quarter to one-half a minute, the loop is dipped in water. The coagulation time is taken at the moment when the blood ceases to be dissolved, but fibrin shreds are demonstrable and some elastic coagulum floats in the water.

Vierordt's (7) first suggestion for determining the coagulation time was extremely crude. When a capillary tube is touched to a drop of blood upon the skin, the blood will flow up the tube. By using a series of tubes and constantly

[1] A verbal communication.

testing, there comes a time when the developing coagulation prevents any more flow. He quickly saw the many possibilities for error in this method and gave it up. He then adopted the method which bears his name. He makes use of carefully cleaned capillary tubes 1 mm. in diameter and 5 cm. long. Touching the tip to a drop, the blood is allowed to flow up until a column 5 mm. long is obtained. Into these tubes is introduced a white horse hair about 10 cm. long. The hairs are previously extracted in alcohol and ether, and washed and dried, to make them as uniform in character as possible. Care must be taken not to handle that portion of the hair which is pushed through the blood, so as to project from the opposite end of the tube. The part of the hair projecting from the tube is not colored red by its passage through the fluid blood. The beginning of coagulation is noted when the fibrin separates and small coagula cling to the hair as it is drawn out. Coagulation is completed when no more new coagula are deposited on the hair. The hair which has been drawn out slowly, will now show a clear colorless portion at both its ends and a section between them covered with red coagula. The time is noted when the blood enters the tube. From this moment until the deposition of fibrin on the hair ceases, is the coagulation time. The average time of 262 observations upon himself was nine and a quarter minutes, with limits of seven and eleven minutes. This is very surprising when one considers the great amount of surface contact, but it is probable that the end point which he notes is a much more advanced stage in the coagulation process than that of other methods. He describes the finest details of obtaining the blood and the precautions to be observed to make the method as constant and the results as equal of comparison as possible. He uses the utmost pains to produce a method of value. He emphasizes such points as the need of obtaining a free flow at the puncture, the difference in drops when comparisons are made, the avoidance of dirt and pressure, the effect of temperature, currents of air, and contact. He realizes the many factors which cause variations, and is not overminute in the determination of the time, noting it only by quarters of a minute, so that small differences may be disregarded.

Vierordt deserves full credit as pioneer in this work of applying to clinical medicine the study of the coagulation time. His list of times in pathological cases was the first published, and his method of determination the first of any importance. It is interesting that this is the method used by Sahli (15) in his researches and the only one described in his textbook (16).

Wright's method with capillary tubes is probably the most widely known and most generally used of any. As first proposed (17), the apparatus consisted of six to twelve capillary tubes, calibrated to receive a blood column of 5 cm., with a diameter of about 25 mm. or 0.01 of an inch. A drop obtained in the usual way from the finger is aspirated into one of these tubes and the time noted when the tube is filled. The site of puncture is now wiped clean and "another drop of blood is pressed out for filling the next pipette." He fills at ¼ to ½ minute intervals. The stage of coagulation is tested by blowing down the tube at regular intervals. The results of testing are characterized as "liquid," "clotting," or "clotted." When the tube is found blocked, coagulation has occurred—"clotted." On the other hand, if the contents can be blown out upon a filter paper, demonstration of a shred of fibrin will distinguish "clotting" from "liquid."

For example:

Tube 1	tested	2	minutes	after	filling	.....	liquid.
" 2	"	3	"	"	"	.....	"
" 3	"	4	"	"	"	.....	"
" 4	"	5	"	"	"	.....	clotting.
" 5	"	4½	"	"	"	.....	liquid.
" 6	"	4¾	"	"	"	.....	"
" 7	"	5½	"	"	"	.....	clotted.
" 8	"	5¼	"	"	"	.....	"
" 9	"	5	"	"	"	.....	"
" 10	"	4⅝	"	"	"	.....	clotting.

So the coagulation time lies between 4 minutes 50 seconds and 5 minutes. The normal time is 2½ to 5 minutes, or rarely 6 minutes.

Wright emphasizes the importance for the accuracy of determinations, that the tubes be of the same size and the ligature about the finger be relaxed at frequent intervals. Six months later (18) he proposes as an improvement that the tubes be heated to 37° C. before filling and kept at that temperature during the process of coagulation. This is accomplished by placing the tubes and a thermometer in the pockets of a flannel bandage, which is fitted tightly about a tin cylinder containing water at the desired temperature. He also advises that the 5 cm. column of the blood be drawn up the tube a little way, in order that the blood should not come in contact with foreign substances at the tube's mouth.

Still later (19) in the same year the temperature was changed from 37°C. to 18.5°C., or half blood-heat for four reasons: 1. Coagulation is slower and therefore more accurate; 2, this approaches nearer to the ordinary ward temperature; 3, and so causes little, if any change in the temperature of the tubes; 4, it is the mean between the freezing point and blood-heat. In an article on the treatment of hæmorrhages and urticaria (20), the normal coagulation time observed at half-blood heat is stated to be 2 to 4 minutes in his standard tubes.

In 1897 (21) there comes another change in technique. To bring the tubes to a temperature of 18.5°C., they are immersed in a tumbler about two-thirds full of water of that temperature, with the butt ends downwards, fitted in India rubber caps. After filling them with blood, they are again immersed in the same water, but with points downward and without the caps, the column of air in the tube preventing the mixture of the water with the blood.

In 1902 (22), in an effort to make "more possible the advance in pathology and therapeutics along lines opened up by coagulo-metrical observations," Wright undertook to simplify the coagulometer, to reduce its cost to a minimum,

and so lead to its popularization. This he accomplished by constructing an instrument which can be made in the laboratory. The standard caliber, a tube 25 mm. in diameter, is approximated by constructing one of such caliber that 5 cu. mm. of mercury occupy a length of 5 cm. in the tube.

The calibration is ingeniously simple. A piece of ordinary glass tubing is drawn out into a fine and insensibly tapering capillary stem. Into the wide end of the tube 5 cu. mm. of mercury are introduced, this amount being measured off by means of a standard 5 cu. mm. pipette, such as is supplied with the Gowers hæmocytometer. Tilting the tube and moving the mercury along will show if there is a portion of the tube where the mercury column will occupy 5 cm. in length,—determined by comparison with a centimeter rule. The upper and lower limits of this are marked with a wax pencil. The mercury is displaced 1 or 2 cm. towards the wide end and these limits marked also, the tube being broken off at the lowest mark.

A series of such tubes are then made ready for use. Touched to a drop of blood, the tube is allowed to fill by capillary attraction until 5 cu. mm. have been obtained, as indicated by the marking. The tube, withdrawn from the blood, is then tilted so that the blood is between the two marks which delimit the portion of the tube of the standard caliber. The time of filling is noted as the beginning point. The tube is then placed point downward in water of the appropriate temperature. The series is tested as before, and the end point looked for is the first evidence of fibrin shreds in the blood when it is blown upon a filter paper. In normal blood this takes approximately 5 minutes.

Later in the same year (23), while applying the method to a study of typhoid thrombosis, a succession of slight pricks were used to obtain fresh blood for each tube, thus avoiding the use of a ligature or pressure and the influence of coagulation in the wound. And for the measurement of the standard volume of mercury, a special form of capillary pipette is devised.

Wright's latest modification has only recently been published (24), and can be understood best by reference to the original article. The method is practically the same, but the tubes are further improved upon. By means of his throttled capillary pipette, one is able to draw up just the right amount of blood with the greatest ease and accuracy. The water bath is used at 37°C., and the normal times reported varied from 1 minute 35 seconds to 2 minutes 10 seconds. This shortening of the time, however, is no improvement, rather a great objection. The almost unavoidable factors which cause variations are numerous, and there must be a limit of error wide enough to cover them. But when the coagulation time itself is but two minutes or under, too much importance may be laid on differences of seconds. The pathological differences and those dependent upon technique in this method are of about the same relative value, which must confuse the results.

The application of this latest method is shown in reports by Wright and Ross (25), Ross (26), Nias (27), and Fox (28).

The Brodie-Russell instrument, and the Pratt-Grützner and Boggs modifications are described fully in Section III. Suffice it to say here that, after a trial of all the methods mentioned, we have adopted Boggs's for our standard, as having the greatest accuracy combined with simplicity of technique.

SECTION II. GENERAL REMARKS.

Are deductions from results of experiments justified? What are we to infer from coagulation? its time, beginning and end point; its variability (daily), and other factors causing this?

It takes but little consideration of all the conditions present in the clotting of blood *in situ* (in the wound) to convince one that those same conditions can be imitated but partially in experiments. The question presents itself therefore, whether or not inferences from the coagulation time in experiments can be applied exactly to the same action of the blood in the body. We cannot say with certainty that when there is a delayed coagulation time a serious hæmorrhage will result, as for instance in an operation. But it has been shown repeatedly that it is difficult to arrest a hæmorrhage where the coagulation time is delayed; on the contrary, the time is short in the patients who bleed with difficulty. The coagulation time of a case of hæmophilia by the Boggs instrument was 16½ minutes, a greatly delayed time. A few hours later the clinical clerk found the greatest difficulty in arresting a hæmorrhage from a lancet and finally had to resort to bandaging the head with pressure on the ear. The hæmorrhage was not completely arrested until the following morning, 12 hours later. In this case, two capillary hæmorrhages from lancet pricks in the lobule of the same ear, on the same day, were arrested easily by contact with a cloth. The coagulation time observed on these two instances was 16½ minutes and 18½ minutes. This, as is evident, did not show definitely that any hæmorrhage would be serious,—and so the first two ear stabs failed to prove themselves; but the delayed coagulation time gave due warning of the possibility, as the third hæmorrhage made apparent. Many cases illustrate this same point. These show the relation between the coagulation in the wound and that *in vitro* to be not a fixed one. The conclusion, however, seems to be justifiable that, if the experimental coagulation time is within normal limits, the blood will clot in a wound with normal rapidity. At least there is no case to show that the blood clotted slowly and with difficulty when the time was not delayed. In the reverse case no sure prediction can be made, but a warning is at hand, which should be respected.

"Coagulation Time" must be accurately defined in order to make experimental times of comparative value. It is the period which elapses from the exit of the blood from the vessels until coagulation has occurred, measured by some one of the various instruments. It has never been applied to clotting in the wound. The time should be counted from the first appearance of the drop to be used, and not from the moment when it is picked off the ear, as is done by many

observers. This beginning point is a constant for all records, which is not the case in any other method of estimation, as the speed of flow of drops is variable. Thus from its constancy, it gives an exact coagulation time. For it is reasonable to suppose that the clotting process begins as soon as the blood appears on the surface of the skin or at the edge of the wound. From a deep puncture a drop large enough to be picked off will form in an instant, but from a superficial puncture or one in less vascularized tissues, the flow is slow and scanty. Coagulation proceeds while the drop is forming; and the added period of skin contact has a marked effect in hastening it. The longer a drop is in contact with the skin surface, the more rapidly it clots when transferred to the instrument. With a freely flowing drop the effect is minimal, and the beginning point is only shortened by a few seconds. But each second of skin contact added, shortens the coagulation time by minutes; *ex.*, L. G. had a coagulation time of 23½ minutes when there was a freely flowing hæmorrhage of 3½ minutes from a deep puncture. When the flow from a second puncture was scanty and lasted but 1 minute, the blood coagulated in 18¾ minutes. At another time: With free rapid flow, the time was 22 minutes; with comparatively slow flow, 15 minutes.

The end point must be more or less arbitrarily decided upon for the different instruments and methods. It should, of course, be clear and definite, always the same, and lend itself to satisfactory confirmation. No attempt was made to mark finer divisions of time than quarters of a minute, thus eliminating the small unavoidable variations as far as possible.

A marked characteristic of the coagulation time is its great variability, as is natural when its manifold factors are recalled. The size and character of the contact surface affects the liberation of the ferment. A drop received upon a vaselined or paraffined surface (29) will not clot at all. Uneven or dirty surfaces cause rapid clotting. With a contact surface of uniform character (glass), the greater its extent, the more rapidly coagulation occurs. This fact accounts largely for the variations in normal time secured by the different instruments. The time is shortest, about 2 minutes, in Wright's capillary tubes, because the amount of contact surface is greatest. For uniformity of character a smooth glass surface is used in all the instruments, whether it be capillary tube, disc, slide, or vessel.

Cleanliness of the skin and the instruments is of prime importance.

A word as to the part of the body from which blood is obtained. Hayem using his own method in simultaneous observations, found that the blood from the ear clotted in nine minutes and that from the thigh in two minutes. With a wide limit of possible error in Hayem's method it is none the less likely that the different character of the skin of the ear from that of the thigh would effect the coagulation time, as the more rough and scaly the skin, the faster is the normal clotting. The superficial blood supply also is not the same in ear and thigh.

It is not possible to make the character of the stab the same each time, but by trying always to obtain a ready flow one reduces to a minimum the factor of skin contact. A deep puncture will give the most uniform flow. Absolute equality of speed in successive hæmorrhages cannot be obtained, due to the difference in the vascularity of tissues of the same and different individuals, to changes in blood pressure, variations in vascular tone, dissimilarity of punctures, and many other inconstant and uncontrollable factors.

One should avoid producing active or passive congestion in any way. Tissue juices affect the time. The admixture of lymph and the increase in carbon dioxide both shorten it.

There is considerable difference in the coagulation time of the different drops of a hæmorrhage. Those at the beginning or the end clot more rapidly than those in the middle, because of the different rate of flow,—more evidence of the influence of skin contact. For this reason and because of the fact that often the flow is slow in starting, the routine is to wipe off the first drop which accumulates and use always the second or third for the experiment. In no case should those near the end of the hæmorrhage, be used.

From the first, observers have noticed the effect of temperature on coagulation. It is well known that cold delays and warmth hastens it. But it is only marked differences in temperature which affect the time, which, as a rule, are not met with in the wards or in the laboratory.

Drafts of air through evaporation, affect the coagulation time in those methods where the blood is directly exposed to them. The drop when the time is slow may dry up before it coagulates, if thus exposed.

Daily variation probably plays a rôle in the coagulation

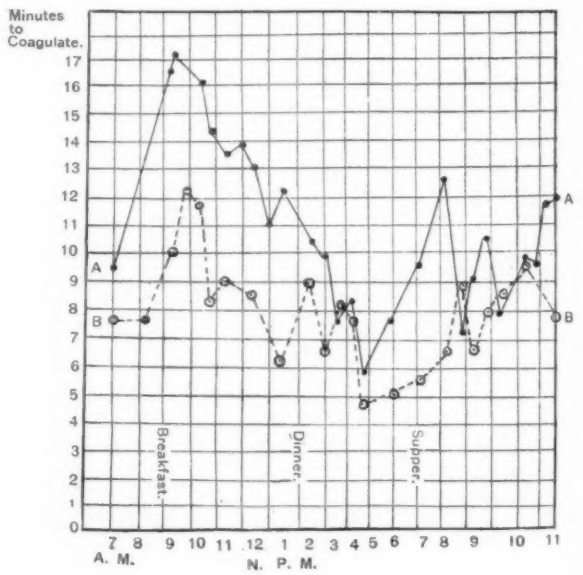

FIG. 2.—Daily curves of A and B.

time. Two curves were made, the authors taking observations every hour upon themselves with blood from the finger

tips. One used the Boggs modification of the Brodie-Russell instrument, and the other the practical modification of Milian's slide method (Section IV). To avoid influencing each other's results, they worked apart. Three interesting features of these studies may here be noted.

First, the fact is striking that their results correspond closely. The longest coagulation time for both A. and B. occurred at about 9 a. m., soon after breakfast. The fall from this was gradual until the shortest time during the day was reached at 4 p. m. After this the course for both was the same—a gradual rise.

The importance of the knowledge that such curves exist is evident. That they should exist and with some degree of constancy seems reasonable. The body activity, intake of food, digestion, sleep, and other physiological processes must have their effect on the body fluids, and they in turn on the coagulability of the blood. In a methodical life these influences would naturally be greater at definite periods of the day. Fuller discussion of these problems must be postponed until more records are at hand.

Second, the correspondence of the author's personal curves with the morning and afternoon observations in Table III, Section III, is noteworthy. These are the results of 251 observations made with the Boggs instrument. It is striking that of the morning records 67.5% were delayed times (beyond 8 minutes). The majority of the afternoon observations were normal times. This resemblance to the daily curves may be merely a coincidence, but it is strange that such a large majority of our cases of delayed times should occur in the early morning and the normal ones in the afternoon.

Third, even if there is no definite daily curve, it is evident that variations do occur during the day. For this reason, in a series of observations, it is important to make them at about the same hour.

As some drug the patient may be taking might alter the coagulation time, careful inquiry should be made as to this point, (see Section V), before drawing any results. The necessity of confirming the result cannot be too much emphasized. This can be done by wiping off the drop with a clean cloth or filter paper. When a definite coagulum is found the test is positive. In such cases only should the record be accepted.

SECTION III. BRODIE-RUSSELL METHOD, BOGGS MODIFICATION.

Principle of the method; the original instrument; a modification by Pratt and Grützner; Boggs modification; relative merits of the three instruments; method of use in detail; the end point; normal time with the Boggs instrument; discussion of lack of uniformity in results of different observers; factors causing variation of particular significance for this method.

The principle on which the Brodie-Russell method of determining the coagulation time of blood is based is very simple. A drop of blood placed in a moist chamber can be set in movement by a very light draught of air tangentially directed. When coagulation has occurred, movement ceases. The process is watched under the low power of a microscope.

The original instrument was devised in 1897 by Russell and Brodie (30). It consists of a deep air chamber, closed below by a glass plate, upon which rests a layer of water. The air chamber is surrounded by a water-jacket, which has inflow and outflow tubes, so that the temperature of the air chamber can be regulated. Into the upper orifice of the air-chamber is fitted a metal ring in which is cemented an inverted truncated glass cone. The water jacket is pierced at one side by a glass tube, which is drawn out to a fine capillary at its inner end and is so directed as to throw a fine stream of air on the edge of the drop of blood.

Pratt (31), assisted by Professor Grützner of Tübingen, devised a modification of the original instrument in 1903. Pratt considered the water jacket and the truncated glass cone unnecessary features, which tended merely to make the instrument more complicated and costly. He omitted them in the construction of his modification without, as he thought, impairing the accuracy of the method. The Pratt-Grützner model is made by fastening a metal ring about ⅞ mm. in height and 1½ cm. in diameter to an ordinary glass slide by means of cement. A hole in this ring admits a small metal tube drawn out to a small orifice at its inner end, and to its outer end is attached a rubber bulb, as in the Brodie-Russell model. The upper free rim of the ring is coated with vaseline. A drop of blood is picked up on a heavy cover slip and the slip then placed on the ring, so that the drop hangs in the moist chamber. The vaseline holds it in place.

Boggs[2] devised an instrument in 1903, modelled after the Brodie-Russell. The water jacket is omitted as in the Pratt-Grützner type, but the inverted truncated glass cone is retained. The metal ring holding the cone has a milled edge, projecting slightly beyond the body of the instrument. (This is a decided advantage over the Brodie-Russell instrument, in which the ring is relatively small with a rounded edge which makes it awkward for handling.) The metal collar fits snugly in the upper opening of the air-chamber and is pierced by a small hole, which allows an equalization of pressure in the chamber when air is blown in by means of the rubber bulb. The metal air chamber is pierced at one side by a metal tube drawn out to a fine hole and adjusted, as in the Brodie-Russell method, so as to throw a fine stream of air at a tangent to the edge of the hanging drop. On opposite sides of the instrument are two metal bars, which are merely for convenience in handling.

Apparently there would seem to be very little choice in the use of any of these three instruments. They are all based on the same principle, as established by Russell and Brodie, namely, that the movement of blood in response to a draft ceases when coagulation has occurred. Practically, however, the Boggs model possesses decided advantages over the other two. It lacks the cumbersome and needless water-jacket of

[2] With his kind permission we are able to give the following description of it, which has not previously been published.

the original instrument, and its construction is altered in other ways so as to make it more practical. It is superior to the Pratt-Grützner instrument because of the retention of a cone, by which a drop of blood of approximately the same size is always observed. In the simple instrument of Pratt there can be no close uniformity in the size of the drop. This is a very serious objection, as there is a great and constant variation in the coagulation time depending upon this factor. The process is progressive and a small drop clots much sooner than a large one. The difference between drops 3 and 5 mm. in diameter is from two to four minutes for normal times, and this difference is increased in the case of delayed coagulation periods. This is well shown in records taken by the slide method, of which the following is one:

TABLE I.

Slide method.			
Size of drop.....	3 mm.	4 mm.	5 mm.
Coagulation time.....	4′	5′	6′ 15″
" "	4′	6′ 30″	7′

This same variation is shown with instruments of the Brodie-Russell type, when the inferior surfaces of the cones have different diameters. The following are simultaneous records taken with three different instruments,—an original Brodie-Russell instrument, cone 3 mm. in diameter, and two instruments of the Boggs type, one with a cone surface of 3.5 mm., the other of 4 mm. in diameter. (No use was made of the water-jacket of the Brodie-Russell instrument.)

TABLE II.

Brodie-Russell Instrument.		Boggs Instrumet.	
Size of drop.....	3 mm.	I. 3. 5 mm.	II. 4 mm.
Coagulation time.....	5′		9′
" "		5′ 30″	7′ 15″
" "		2′ 45″	5′ 15″
" "		9′	13′ 30″

The above tables, I and II, show definitely that the size of the drop is a very considerable factor in the length of the coagulation period. In the Pratt model no means are taken to make this factor constant. Its limit of error, therefore, is more than in the case of an instrument with a cone, which picks up a drop of uniform size. With cones of the same size, however, a series of successive observations upon the same individuals ought not to vary more than half a minute.

The technique in general is the same for all methods of determining blood coagulation, and has been fully discussed under General Remarks, Section II. The beginning point should be the first appearance of the blood from which a drop is to be taken for observation. The blood is allowed to well out at the puncture in the form of a small globule, and the inferior surface of the cone is touched lightly to it. The flat surface of the cone must be touched to the drop and care taken not to smear the edges of it. It is very simple to get a drop the exact size of the cone surface. When the glass is approached close enough, the blood seems to spring up and flow just to the edges of the disc. However, this will not occur upon a dirty cone surface. If the sides of the cone are smeared, or the drop fails to completely cover the cone surface, the latter must be carefully cleaned and dried. After the old drop is wiped off, time is taken on the first appearance of blood again, and another attempt made to pick off the right sized drop. It may be necessary to make a new puncture, as one should be careful not to use the late drops in a hæmorrhage.

With the drop on the cone, the latter is fitted quickly in the moist chamber. The apparatus is placed under the low power of a microscope and the motion of the blood caused by blowing with the rubber bulb is watched. As this is the most objectionable feature in the method, it should be made as uniform as possible for all records. Very light blowing, which is all that is required, at long intervals, minimizes the weakness of this method. Only the periphery of the drop need be watched. Here it is thinnest and clotting begins and ends first. Because of its thinness, the process can be better seen and the changes are more definite.

The changes in the movement of the red blood corpuscles set up by the blowing may, for the convenience of description, be divided into four stages:

1. Rotation of the drop as a whole or free flowing movement of the corpuscles individually. Fig. 3, *A*.

2. Cohesion of corpuscles and flowing movement in clumps and groups. Fig. 3, *B*.

3. Marked elasticity, flowing having ceased; a vibratory, elastic, *circular* motion. Fig. 3, *C*.

4. Elastic *radial* motion; indentation of the edge, the first appearance of which *radial* elasticity is the end point. Fig. 3, *D*.

Early in the process very light blowing starts the drop rotating as a whole, or causes a stream of corpuscles to flow about the periphery. The corpuscles move along individually and show no tendency to clump, Fig. 3, *A*. Gradually this movement presents a sticky, gelatinous appearance. The corpuscles begin to move in clumps and groups with clear, translucent spaces intervening, Fig. 3, *B*. In some cases a thin rim of corpuscles adhere along the periphery of the cone, probably the result of evaporation. This seems to have no particular influence on the end point, or the length of the coagulation period. Almost imperceptibly the drop takes on a definite elasticity. The flow of corpuscles becomes increasingly sluggish. Grouping and translucency disappear gradually and the corpuscles begin to move with a definite relation to each other. Elasticity becomes more and more marked, and flowing ceases completely. The corpuscles move in a *circular* direction when struck by the tangentially directed stream of air, but quickly recoil to their old positions. The movement is *circular* and elastic, Fig. 3, *C*. The drop should be carefully watched when this *circular* elasticity appears. In from 30 seconds to 7 to 8 minutes, as the time is short or delayed respectively, the final change occurs. This is marked by the appearance of a definite *radial* elastic movement. Different parts of the periphery should be watched, as this *radial* movement does not appear everywhere at once. *Circular* elasticity still persists, but the two are easily distinguishable. When

struck by the stream of air, the corpuscles, due to their *radial* elasticity, move as a gelatinous mass a little way towards the center of the drop, and quickly spring back into their old places. There may be at the same time a small indentation at the point where the corpuscles recede, which immediately fills in on the rebound, Fig. 3, *D*. The first appearance of this movement marks the end point in the determination. The time is taken at once, the cone of the instrument immediately removed, and an attempt made to pick off a clot from the cone surface with a cloth or piece of filter paper, as confirmation that coagulation has occurred. A firm visible clot can be picked off when *radial* elastic movement has been observed. Fig. 3, *E*, pictures a "vicious circle," which is merely a whipping of the blood from too vigorous pressure on the bulb, and here the end reaction fails to appear.

The end point is largely a matter of degree in the process of coagulation. The appearance of *radial* elastic movement does not indicate that the whole drop has clotted. It is merely one of the stages in the process; but it is of such definite and uniform occurrence as to form an almost ideal stage for observation, and for conformation a visible clot can be picked off where the definite elastic movement has occurred. In case there is no clot, a second observation should be made, as there may have been some error in judgment of the end point of the first record. In our studies, we have paid particular attention to this point of confirmation. In our early records the first appearance of the *circular* elastic movement was taken as the end point of coagulation. In no one of these cases could a confirmation be made by the presence of a clot. This stage forms a poor end point for other reasons. It is much more gradual in occurrence and is less definite, and allows greater variations in judgment. In some further observations, removal of the cone after the appearance of *circular* elasticity, but before *radial* movement had occurred, failed in most cases to show the least visible macroscopical clot. Such instances were most striking in the case of delayed times. This fact, therefore, that with the appearance of *radial* elasticity it is possible to demonstrate a macroscopic clot, is of importance, as it furnishes a constant technique, the results of which are strictly comparable.

Observers who have used the Brodie-Russell method have taken various portions of the different stages in the process as their end point. Pratt (31) took the moment when the individual movement of corpuscles ceased. He used no means of corroboration. Murphy and Gould (5) judged as an end point "the moment when the edge of the drop fails to rotate in response to the air current. As a control in all cases the cover glass was removed and the blood pricked with the point of a needle. Demonstration of fibrin shreds was regarded as a final evidence that coagulation had occurred." Brodie and Russell (30) considered the blood coagulated "as soon as a rim at the periphery is solid, and blowing simply indents this rim, without causing rotation." Pratt, Murphy and Gould probably used that stage which is labelled *C* in Fig. 3,—the *circular* elastic movement. Brodie and Russell very likely considered the *radial* elastic movement as the final change. This, with other considerations, may explain the inability of these different observers to secure a uniform average time.

It is only possible to approximate a normal time. The mean of a large series of records can be taken as the normal. Many individuals apparently in good health have delayed times. The majority of the records in a large series, taken on all sorts of cases, will be within normal limits. By tabulating such a series in columns of time divisions, it is possible to establish the limits for a normal time. The column summing up the greatest number of cases would reasonably be supposed to represent the mean normal time for the series. The average of the whole series of delayed, normal, and rapid times would be much higher than the limits established by such a table, and should not be considered as normal.

The following table was constructed from a series of 251 records, all taken with a Boggs instrument having a cone surface 4 mm. in diameter. Each record tabulated was confirmed by the presence of a macroscopic clot.

TABLE III.

Time of Day.	Time of coagulation in minutes.										Total.
	–3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11+	
A. M. 8-10....		1	3	3	4	4	4	7	5	15	46
" 10-12....	1	5	7	2	6	4	1	3	1	7	37
P. M. 12-2....		1	8		4	1	2	1		3	15
" 2-4....	2	2	4	6	2	2	3	2		4	27
" 4-6....	8	15	14	18	12	13	8	6	5	6	105
" 6+....		1	2	6	2	5	2		1	2	21
Total.....	11	25	33	35	30	29	20	19	12	37	251

163 of the records have times below 8 minutes, and, although more than half of the series are pathological cases, there are only 88, or 31%, with times above 8 minutes. The average time for the 251 records is about 9 minutes. But 37 of the records have a time of 11 minutes or above. The longest time in the series is 33 minutes. The mean of the 37 delayed times is 21 minutes, and this considerably raises the average for the whole series. Leaving them out, the remaining 214 records give an average of 6 minutes and 40 seconds. It is fair to conclude that records below 7 to 8 minutes are normal, while coagulation periods passing this limit are proportionally delayed.

The results of other observers are interesting. Pratt found an average time of 4 to 5 minutes. Murphy and Gould, from 300 observations, give an average time of 3 minutes 12 seconds, with limits of 3 to 6 minutes. Brodie and Russell's observations were made with reference to the influence of temperature and they publish only a few records. At 20°C., the average time on normal individuals was 7 to 8 minutes. Generally speaking, all agree that an average time for the Brodie-Russell instrument is between 3 and 8 minutes. The reasons for the differences are mainly two. In the first place, as has already been stated, all the observers have not used the same stage in the process as end point. Brodie and Russell, who used a late stage, record a much longer time. In the second place, it is very unlikely that all observers have

used the same sized drop. Again, the beginning point may have been different.

The general factors that influence the length of coagulation have been mentioned, but there are three that are of particular importance in the use of the Brodie and Russell method. These are, the size of the drop, the amount of blowing on the drop, and the stage in the process that shall be taken as the end point.

The question of the size of the drop is purely arbitrary, so long as neither extreme is used. The point of importance is that all instruments of this type should have uniform-sized cone surfaces. Records taken with instruments the size of whose cones vary, are valueless for comparison. There were three coagulometers in use at the Johns Hopkins Hospital. No two of them had cone surfaces of the same diameter. One was a Brodie-Russell instrument, and the other two were of the Boggs type. The diameters of the cone surfaces of the Boggs instruments were 3.5 and 4 mm. respectively, and of the other 3 mm. Table II shows the discrepancies in the results of simultaneous records by these three instruments. A 4 mm. drop is a good mean, and has been taken as the standard for our records.

The blowing on the drop is the one great objectionable feature of the method. This increases air contact, causes more rapid evaporation, and very likely has a considerable influence on the length of the period. If the factor were constant, the objection would be largely obviated. The observer can overcome this satisfactorily by using very light pressure on the bulb at long intervals. Continuous blowing should not be used, and the interrupted periods of blowing should be only momentary. The force of blowing can be easily judged by watching the drop.

The question of end point has already been discussed.

Section IV. A Practical Modification of Milian's Slide Method.

Reason for adoption of modification; size of drop the essential feature; selection of 3, 4, and 5 mm. sizes; change in contour on tilting; coagulation complete when no change in contour; confusion arising from presence of serum exudate; adoption of standard size drop; method of tabulation of records; time averages; comparison of results with Boggs method; delayed time and evaporation; normal time by slide method; practicability.

Having determined the inaccuracy of Milian's method, the following modification was tested.

Ordinary glass slides were used, though any glass surface will do. The ear was punctured in the usual way, the first drop wiped off and time counted from the appearance of the second drop. By lightly touching the under surface of a slide to this globule, drops of blood may be picked off for observation. The diameter of the drop picked off depends mainly upon the size of the globule on the ear. It is of advantage to hold the lobule of the ear out, so that the slide may be horizontal when touched to the drop. The drops being upon the under side of the slide, it is turned quickly to prevent them from flowing. Placed over a scale, those drops of the required size are chosen and the rest wiped off. Coagulation is judged as by Milian's method. It is complete when tilting the slide will cause no change in the contour of the drop.

The size of the drop is the essential feature of this modification. Milian allowed the blood to drip from a puncture wound upon the surface of a slide, and the drops were usually about 1 cm. in diameter. The size of the drop obtained by touching a slide to a globule can easily be controlled. Usually three to four drops were picked off on one slide, and as a rule two to three slides were used. When the drops were measured on a millimeter scale, only those approximating 3, 4, 5, and 6 mms. in diameter were saved for observation. The others were wiped off to avoid confusion. Where so many drops are used, it is necessary that time be taken on the first appearance of each new globule on the ear. So there are usually two or three different beginning points for the records and the slides should be numbered or kept in the order of these beginning points. Particular care was taken in all our records to have one 4 mm. drop for comparison. A number of simultaneous observations were made too with the cover-slip hanging drop method.

There are two ways of watching these drops to determine when coagulation has occurred. The contour of the drop may be watched from the side. In this case the appearance before coagulation will be as shown in Fig. 4, *A*, and after coagulation, as in Fig. 4, *B*.

Another way is to hold the slide to transmitted light and judge if there is a flow in the drop by the changes in density. The denser portion is in the center when coagulation has occurred, and at the lower edge according as the slide is tilted, when the drop is still fluid. Fig. 5, *A* and *B*.

It is well to combine these two methods as an aid in judgment of the end point. As a rule it is easy to judge when coagulation has progressed far enough to prevent a change in the contour on tilting the slide. Each observation should be confirmed by picking up a macroscopic clot with the tip of a cloth or filter paper.

The 4 and 5 mm. drops are the best adapted for this method. In the smaller ones the contour changes are difficult to judge, and the larger ones meet with the same objections, as in Milian's method. The 4 mm. drop gives a definite end point, except for very long times. In delayed times, evaporation is such an important factor that the 5 mm. size gives more satisfactory results. The 4 mm. drop may be so nearly dried up as to prevent the judgment of an end point. It is well, therefore, to always get a 4 and a 5 mm. drop for observation.

Tables IV and V are made from simultaneous records with the Boggs and slide methods. In the first table all four sizes of drops were observed; in the second, only the 4 and 5 mm.

TABLE IV.

	Slide Method.					Boggs Method.
					Average.	
Size of drop.....	3 mm.	4 mm.	5 mm.	6 mm.	4-5	4 mm.
Time in minutes....	3½	4¼	6	8¼	5½	5½
" " "	4½	7	9	12½	8	7¼
" " "	9¼	11½	13¾	17½	13	

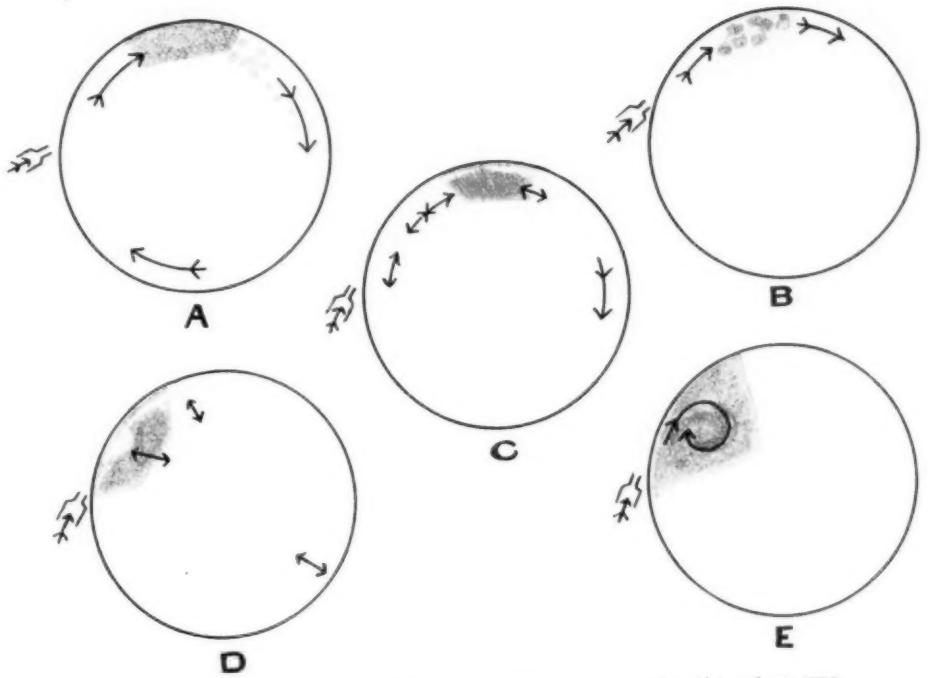

FIG. 3.—Stages in the coagulation process as seen under the microscope.

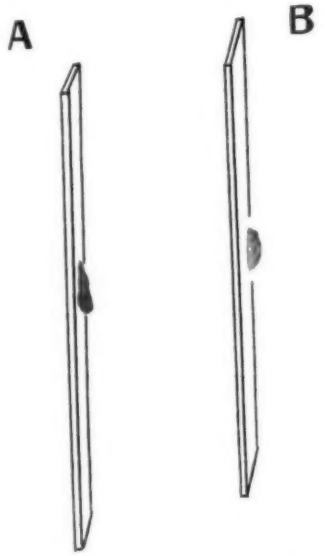

FIG. 4.—Contour of the drop before and after coagulation.

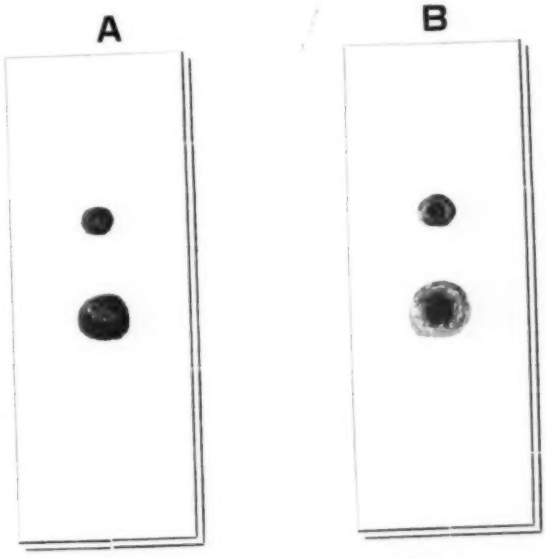

FIG. 5.—Same by transmitted light.

TABLE V.

	Slide Method.			Boggs Method.
Size of drop.....	4 mm.	5 mm.	Average 4–5	4 mm.
Time in minutes.....	6	8¼	7½	7
" " "	6¼	8½	7¼	6¾
" " "	6½	9½	8	8
" " "	5½	9½	7½	9
" " "	6¾	8¾	7½	8
" " "	6½	8	7¼	8
" " "	5¾	9½	7½	8¼
" " "	5¼	6¼	5¾	6

From the above two tables it is seen that the averages correspond very closely to the times obtained with the Boggs instrument. The time by the Boggs method seems to range between that of the 4 mm. and 5 mm. sizes of the slide. The 4 mm. drop upon the slide is exposed and naturally clots more rapidly than the 4 mm. drop protected in the moist chamber of the Boggs instrument. But a 5 mm. drop coagulates more slowly. So 4 mm. and 5 mm. drops are taken, and the mean of their coagulation times is what we term the *time average*. This lends itself most favorably to comparison with the results of the Boggs instrument.

The slide method as a quick, convenient and practical means of determining the coagulability of the blood is thoroughly reliable, and can be used in the laboratory or sick room. Its simplicity is the greatest point in its favor. The fact that the whole apparatus is but a clean glass slide and a millimeter scale permits everyone to use it.

The chief objection is the exposure and consequent evaporation. This apparently has little effect on the result in those cases where the time is within normal limits, and is no more objectionable than the blowing in the case of Brodie and Russell's method. But where the time is very much delayed, evaporation has so much better chance to modify the result that this is very often unsatisfactory. Besides the evaporation, the mechanical tilting hastens the time.

The records in the following table show that the method is of practical value even in delayed times.

TABLE VI.

	Slide Method.			Boggs Method.
Size of drop.....	4 mm.	5 mm.	Average 4–5	4 mm.
Time in minutes.....	8	9½	8¾	11½
" " "	7¾	8¼	8	11¾
" " "	7½	10¾	9	12
" " "	...	(1)12½	...	12
" " "	...	(2)10¾	...	....
" " " 13		15¾	14	14
" " " 13		...	...	15½
" " " 14½		15¼	14¾	14½

The average time for the slide method, taking the mean of the 4 and 5 mm. drops as the record for a case, is practically the same as that for the Boggs instrument with a cone surface of 4 mm. The great majority of the records fall between 5 and 8 minutes. Anything above 7 to 8 minutes is delayed. Below 8 minutes a record would be within the limits for a normal coagulation time.

In an attempt to determine the time significance of evaporation as a factor in the coagulation process, the following modification of the simple slide method was resorted to. Hanging drop slides used in bacteriological work were smeared with vaseline about the rim of the well. The blood for observations was then picked off on ordinary cover slips and these placed over the well, so that the drop would be in a closed chamber. The vaseline holds the slip in place, prevents its moving when tilting the slide, and seals the chamber. The end point for the drop was determined in the same way as for the plain slide. Judgment of this was, however, somewhat more difficult, as the contour could not be seen so clearly and the determination had to depend almost wholly on density changes, as shown in Fig. 5, *A* and *B*. In each case simultaneous records were taken by Boggs' instrument and by the slide method.

The results may be briefly tabulated as follows:

TABLE VIII.

Boggs Method.	Slide Method.			Cover-slip Method.		
4 mm.	4 mm.	5 mm.	Average.	4 mm.	5 mm.	Average.
11½	8	9½	8¾	11¾	....	....
7¾	5½	6½	6	7¾	....	....
6	4¾	6½	5½	6	....	....
7¼	8¼	10	9	9	....	....
8	6½	9½	8	9	....	....
10¼	8¾	10	9¼	9¼	....	....
8¼	5½	6½	6	9	10½	9¾
8¼	6¾	...	...	8¼	....	....
9¾	...	10	...	...	12	....

It is seen from the above table that evaporation, or open exposure, is a factor of considerable importance in hastening coagulation. The times in the 4 mm. column of the cover-slip series correspond more closely to the records of the 4 mm. drops of Boggs method than do the figures in the 4 mm. column of the slide method. As a rule they tend to be longer than the Boggs records, and this is probably due to the fact that the blowing on the drop, by the Brodie-Russell scheme, exerts an influence on the clotting period that is not present in the cover slip. Each have closed chambers, but the end points are very unlikely to correspond exactly, as the methods of determination are so different. It is fair to assume, therefore, that the difference in the times of the 4 mm. drops for the two slide methods is very largely due to the more rapid evaporation in the case of the unprotected drop. The difference is slight, and inasmuch as the simple slide is so much more convenient, the cover-slip method was used merely to demonstrate the point in regard to evaporation.

SECTION V. COAGULATION TIMES IN PATHOLOGICAL CASES, AND CALCIUM THERAPY.

I. Pathological cases, icterus; typhoid fever; urticaria; purpura; hæmorrhages; anæmias; aneurisms.

We have considered:

(a) Catarrhal jaundice.

(b) Jaundice due to cholelithiasis.

(c) Jaundice due to malignant disease.

The cases of catarrhal jaundice have shown a normal coagulation time, 5½ to 6 minutes. (All the records are taken with the Boggs instrument.) In jaundice due to cholelithiasis, the times range from 5½ to 12½ minutes, with an average of 8 minutes. This is on the upper limits of normal and is in contrast to jaundice due to malignant disease, where the average is 13¼ minutes, and the limits 9 to 20½ minutes.

For typhoid fever Wright (32) has claimed a delayed time during the acute stage and a short time in convalescence, and he lays stress upon the occurrence of hæmorrhage and thrombosis in connection with this. Our cases have shown the difference, but it has not been marked. In contrast to an average of 7 minutes in convalescence is that of 9 minutes in the acute stage. Some individual cases have been more striking:

L. C.—12th day, 12 minutes; 23d day, 7½ minutes; 31st day, 3 minutes.

J. L.—15th day, 13¼ minutes; 21st day, 9¼ minutes; 44th day, 5¼ minutes.

None of the cases with hæmorrhage have had a delayed time, and all of them have shown an increased coagulability after the hæmorrhage.

In the typhoid thromboses, the longest time was 6½ minutes, the average 5½ minutes. The majority of our cases have been on a milk-albumin diet, and the few fed more liberally showed no differences from the general group.

Four cases of hæmophilia had times ranging from 11 minutes to 33 minutes. Two of them were brought down to 5 and 6 minutes with calcium:

J.—12 minutes, 6 minutes after calcium.

Dr. B's Case.—30 minutes, 5 minutes after calcium.

The times in urticaria have been variable. Some have been as delayed as 9½ minutes. Some of the cases with diminished coagulability are greatly benefited by calcium. Cases of purpura, hæmaturia without known cause, epistaxis and various hæmorrhages more profuse than usual, have shown times varying from 6 to 9¼ minutes. In this class calcium is of great help.

In the primary anæmias no markedly delayed time has been found. One case of pernicious anæmia had a time of 9½ minutes. The majority range from 4½ to 7½ minutes. We have observed no case of leukæmia with hæmorrhage. An acute lymphatic leukæmia without hæmorrhages had a time of 8¼ minutes. The blood in two very severe secondary anæmias,—a case of hæmaturia and of typhoid fever respectively,—clotted in 14¾ and 14 minutes. As a rule they were not different from the primary cases.

Finally, we have observed many aneurism cases, with the idea of increasing the coagulability of the blood. Never have we found them to have delayed times.

II. Calcium therapy; in cases of normal coagulation times; in those of delayed times; form of calcium; loss of effect after too long administration. Will calcium cause a delayed time? Course of increasing doses. Action of citrates; how to give them; effect of calcium plus citrates.

Calcium therapy.—Calcium therapy in relation to the coagulation of blood is a subject of growing importance and interest. It is generally well known that calcium salts given by mouth, per rectum, subcutaneously, or intravenously, tend to shorten a delayed coagulation time; but the fact that the effect of any dose wears off after continued administration is not so well known, nor is it understood how a delayed time may be reduced and maintained as such. It is too frequently the case that calcium is ordered to assist in the control of a hæmorrhage, when no attention at all has been paid to the coagulation time. The first requisite is always a determination of the time. A normal time is not a contraindication to calcium in all cases. The increased calcium content of the blood may be of aid even when the time is normal. We have found that a normal time can be further shortened. For instance:

H. S.—Aneurism, 7½ minutes; 3 minutes after calcium.

L. N.—Aneurism, 5½ minutes; 3¼ minutes after calcium.

Same case again, 7 minutes; 4 minutes after calcium.

However, it may do harm in some cases. Take for example a case of intestinal hæmorrhage in typhoid fever. The bleeding is due to the erosion of a vessel, and it has never been our experience to find a delayed coagulation time in these cases. In addition a hæmorrhage of considerable size will in itself shorten the time. And yet, with great regularity, one finds calcium ordered after the hæmorrhage, often the only thing the patient is getting by mouth. Whether too much calcium causes thrombosis or not, as Wright claims, there appears to be at least a possibility of this.

Having found the time delayed, calcium is indicated. The lactate is the best salt, as Boggs (33) first pointed out. The chloride is too irritating. We have never seen the lactate cause the slightest gastric disturbance. A good way to give it is in suspension in simple syrup, or the powder or capsules may be used. A solution of the chloride in normal salt may be used for infusion intraperitoneally or intravenously, but subcutaneously it may cause great sloughing. One of our cases was complicated by a breast abscess with calcification (34). It has been suggested that the lactate is split in the stomach and the calcium absorbed as a chloride. If so, the best time for the administration would be after meals. Wright has claimed that too much calcium will lengthen the coagulation time, more even than it was previously. Our experience has been as a rule that when one does continue it too long, the effect wears off and the time returns to about what it had been previously. We have but one case in which possibly the calcium itself lengthened the coagulation time to more than it had been before it was started. It is:

8.—Malignant Jaundice.

Coagulation time.	Date.	Calcium lactate.
9¼ minutes in a. m.....	Apr. 13	
	Apr. 14	gr. 80 q. d. begun.
	Apr. 15	continued.
	Apr. 16	gr. 120 q. d.
	Apr. 17	gr. 160 q. d.
8 minutes in a. m.....	Apr. 18	continued.
	Apr. 19	gr. 240 q. d.

Coagulation time.	Date.	Calcium lactate.
17 minutes in p. m.....	Apr. 20	gr. 480 q. d.
20½ minutes in p. m.....	Apr. 21	Calcium discontinued.
	Apr. 22	
11¼ minutes in a. m.....	Apr. 26	gr. 10 at 8-12-4-8.
7½ minutes in p. m.....	Apr. 26	
6¾ minutes in p. m.....	Apr. 28	

Our idea is that in the gap of four days without a record, the coagulability was increased to normal, and was then delayed again by too much calcium. As the progressive increase in calcium kept up, the time became more and more delayed. When the intake stopped, the time became shorter, as the body rid itself of the excess calcium. Later, the time was reduced to normal by a small dose of calcium lactate.

As a rule, however, the type of cases is as follows:

M. H.—Jaundice. Cholelithiasis. **Was receiving calcium lactate gr. 80 q. d. before first observation.**

5½	minutes in p. m.....		1st day.	
4½	"	"	2d day	
8	"	"	4th day	increased to gr. 120 q. d.
7½	"	"	5th day	
5½	"	"	6th day	
6	"	"	7th day	
9	"	"	8th day	

F.—Aneurism. **Calcium was given to increase the coagulability and induce clotting.**

5½	minutes....	1st day	Calcium lactate gr. 45. q. d.
3½	"	2d day	
3¼	"	3d day	
7	"	5th day	Calcium increased to gr. 90.
4	"	7th day	
7	"	11th day	

A. S.—Jaundice. Cholelithiasis.

9½ m. before Calcium.
6¼ m. on 2d day of Calcium.
4 m. on 4th day of Calcium.

I. B.—Malignant Jaundice.

11	minutes.....	calcium.
9	"	
19	"	increased calcium.
14	"	
9	"	

These typical cases show how the calcium increases the coagulability, but can maintain it only about three to five days. If the dosage is then increased, the time is again shortened.

In order to reduce a delayed coagulation time and maintain it as such, it is better to begin with a small dose, as gr. 10, four times during the day. Daily determinations are made, and when the time is becoming delayed again, increase the dose to gr. 20 q. 4 h., then to gr. 30 and gr. 40. As a rule, the small increases will fall due every 3 to 5 days, and will succeed in maintaining a short coagulation time. Large amounts of the drug are inconvenient to take. So we usually go no higher than gr. 40 q. h. After a free period of 3 to 5 days, the effect is gained as before, in beginning a new course. This fact suggested a second method of giving a small dose for 3 to 5 days and following with a free period of the same length, thus alternating as long as necessary.

In contrast, the citrates are supposed to have the opposite effect, namely, to delay a short coagulation time. Our experience is limited, as we found very few cases where a delayed time was desirable. It would seem, however, that their effect wears off in a manner analogous to that of calcium; that to maintain a delayed time, there must be small increases in the dose at intervals.

The question arose as to whether the citrates might neutralize the effect of the calcium if given at the same time. L. G. was receiving potassium acetate and citrate gr. 15 q. 4 h. as a diuretic. On the third day she had an epistaxis of 400 cc. Calcium lactate was given, gr. 15 q. 4 h., and increased to gr. 30 on the seventh day. Both were continued for 9 days, during which time the coagulability of the blood remained diminished. Times of 10, 10¼, 9¼, and 8½ minutes were obtained. The first normal time, 7 minutes, was on the day after the citrates were discontinued. The evidence in this one case points to the citrates acting as a check upon the calcium.

We then made use of a case of erythema nodosum, as an experiment in this line. The chart explains itself.

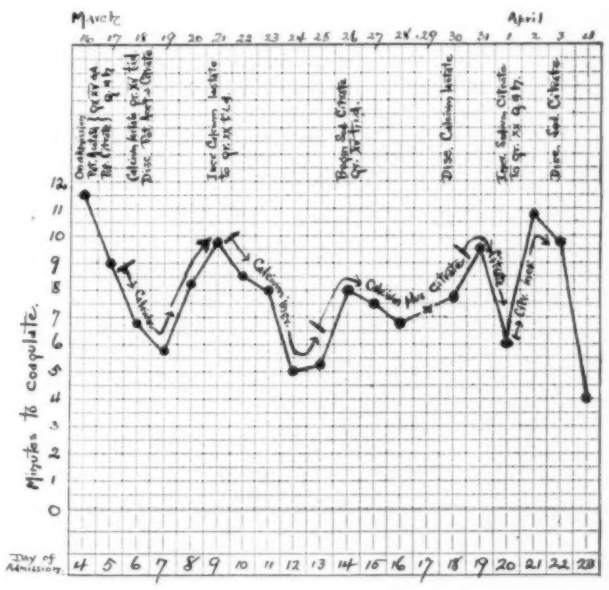

FIG. 6.—A. G. Erythema nodosum.

The delayed time was reduced with calcium; its effect wore off, and it was a second time reduced. Then with a time of 5¼ minutes the citrate was begun. For five days the two together maintained a fairly constant time between 7 and 8 minutes, which is about a mean between the extreme effect of the calcium, 5 minutes, and that of the citrate alone, 10¾

minutes. Then with the citrate alone, its effect soon wore off, but was renewed by an increase in the size of the dose.

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ON WRIGHT'S METHOD OF MEASURING THE ALKALINITY OF THE BLOOD.

A PRELIMINARY NOTE.

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In the course of certain investigations on methods of measuring the alkalinity of the blood now being pursued in this laboratory under the direction of Dr. Thomas R. Boggs,[1] we have had occasion to test the value of the method described by Sir A. E. Wright (1); and we have considered it of interest to give a preliminary report of our results, discussing only the practical technical value of the method, and not attempting, for the present, to deal with the more complex chemical questions involved.

The method as described is as follows: Blood is drawn from a finger into a capsule, one or both ends of which are sealed. It is then allowed to stand for from 3 to 24 hours. This is done, because, as shown by Zuntz (2), Peiper (3), and Winternitz (4), the alkalinity of the blood diminishes rapidly until coagulation occurs, after which it remains fixed. When the blood has stood until a condition of constant alkalinity is reached, it is titrated. The capsule may, however, first be centrifugalized to further separate the serum from the clot. The titration is by means of capillary pipettes like those used in the opsonin work. Solutions of normal sulphuric acid, diluted 20-, 30-, 40-, 50-, and 60-fold are prepared. The titration is against delicate red litmus paper, prepared by acidifying very fine (Grübler) neutral litmus paper in a solution of 1:20,000 hydrochloric acid, repeatedly washing with distilled water, and then drying in an oven at 120° C. Such paper should show an alkaline reaction to normal blood serum diluted forty times. The paper should be glazed or made as smooth as possible by rubbing with a glass rod.

The acids and indicator being ready, a pencil mark is made about 2 cm. from the end of the pipette to be used. Serum is drawn from the capsule into the pipette almost up to the blue mark, leaving an air-bubble index. Then an equal volume of $\frac{N}{20}$ sulphuric acid is sucked in, and the serum and acid are mixed by blowing them into a watch glass and respirating. A drop of the mixture is then put on a strip of the litmus paper. If this is still acid, the same procedure is instituted with equal volumes of serum and of $\frac{N}{30}$ sulphuric acid, and so continued down with $\frac{N}{40}$ acid, etc., until the mixture shows an alkaline reaction. When this is obtained, say with equal volumes of serum and $\frac{N}{40}$ acid, an intermediary solution of $\frac{N}{35}$ acid is made from equal volumes of $\frac{N}{30}$ and $\frac{N}{40}$ acid and the titration is repeated with this dilution. The acid dilution first neutralizing the serum is considered as the measure of the alkalinity, and the alkalinity is expressed in fractions of normal acid; for example $\frac{N}{35}$, $\frac{N}{40}$, etc. No attempt is made to titrate with acids varying by less than a five-fold dilution. Wright found that in a series of 13 normal persons the serum possessed an alkalinity varying from $\frac{N}{30}$ to $\frac{N}{45}$, or an average of $\frac{N}{35}$. In the author's words, this means that the "alkalinity of blood under ordinary conditions of health is such that the addition of one volume of a normal acid, 35 times diluted, to an equal volume of serum, just suffices to deprive that serum of its power of blueing sensitive litmus paper."

Wright advocated this method, not as an accurate measure of the nature or amount of the substances producing the alkaline reaction, but as a convenient approximate estimate of the total alkalinity of the circulating blood. In using *serum* we obviously only make a partial calculation of the total alkalinity, for a great part if not the greatest part of the alkaline reaction is due to the cellular constituents of the blood. Drouin (5) has shown that changes in the alkalinity of the circulating blood can be determined by measuring changes in the serum; and Zuntz (*l.c.*), Peiper (*l.c.*), and Winternitz (*l.c.*) have proved that the alkalinity after coagulation remains constant. Wright considers that his own experiments confirm these results. These facts being demonstrated, the principles underlying the use of serum are satisfied.

A brief survey of the extensive literature on hæmalkalimetry will convince one that a completely satisfactory clinical method has not yet been proposed. It is claimed for Wright's method that it has the advantages of simplicity of technique, that it obviates the necessity of diluting the blood, and that it overcomes the manifest physical difficulties associated with titration of a fluid containing red blood corpuscles. Therefore, if the method fulfills its promise of sufficient accuracy for comparative studies, it should be of clinical value.

Although Wright in his original article only gives the results of estimates of the normal alkalinity, he later (6) showed by this method that scurvy was an acid intoxication, and that in this disease the blood alkalinity was represented

[1] I desire here to acknowledge my great indebtedness to Dr. Boggs for his kind assistance in directing this work.

by values of $\frac{N}{90}$ or $\frac{N}{100}$. Luff (7) considered the method satisfactory for measuring the alkalinity of the blood in gout. Moore and Wilson (8) object to the use of litmus paper as an indicator.

We have used the method as described; but have made it a rule to take blood from patients at practically the same hour, 11 a. m. to 12 m., so as to render constant any effects due to digestion, and have uniformly made our readings three to four hours later. Also instead of the series of watch glasses, the water color palette has been employed to hold the acids and for mixing. Excluding all preliminary experiments in acquiring a constant technique, we have made 55 readings on a total of 37 individuals, including normal persons and patients afflicted with various diseases, such as the acute fevers, diabetes mellitus, tuberculosis, infectious arthritis, cirrhosis of the liver, nephritis, leukæmia, pernicious anæmia, and malignant neoplasm. In most of these diseases rather complete studies have been made by various investigators using different methods, and although the exact quantities given differ according to the method employed, there is a fair unanimity in the general results. We therefore have a basis on which to compare our results.

In two cases we got very low values: $\frac{N}{100}$ in a case of pernicious anæmia just before death, $\frac{N}{90}$ in a case of acute lymphatic leukæmia. The latter case had been followed for six days, from the day of admission to the hospital to death. On the day of admission the alkalinity was represented by $\frac{N}{60}$, while on the sixth day, just before the *exitus lethalis*, it was $\frac{N}{90}$. It is interesting, however, and rather important to note that both these cases presented such striking symptoms of acidosis that the diagnosis of "acid intoxication" was made by the visiting physician on inspection of the patients as they lay in the ward. Also the urine in both cases contained acetone bodies. One other case of pernicious anæmia showed an alkalinity varying from $\frac{N}{40}$ to $\frac{N}{50}$, while several cases of striking secondary anæmia gave values never lower than $\frac{N}{40}$. One case of jaundice, with bile-tinged blood serum and marked cachexia, gave by this method values never below normal, although it is generally agreed that both jaundice and cachexia are associated with reduced alkalinity. We had no cases in which the alkalinity was higher than $\frac{N}{30}$. Two cases of diabetes mellitus, without symptoms of acid intoxication and without acetone bodies in the urine, gave normal readings. Two cases of cirrhosis of the liver, studied from day to day, occasionally showed an alkalinity of $\frac{N}{50}$, but on most days it was $\frac{N}{40}$, and as $\frac{N}{40}$ is normal, we do not feel justified, for reasons to be shown later, in drawing conclusions from such an inconstant slight reduction. Most conditions showed alkalinity within the "normal" limits.

Very early in our investigations it was found advisable to eliminate subjective influences as far as possible. To this end, we took on the same day blood from different patients, almost always including one normal, had the capsules listed and renumbered by a colleague, and then estimated the alkalinity of the various sera without knowing to which individual each capsule belonged. The relative insensitiveness of litmus in the presence of carbonates was a constant source of trouble in the exact determination of the saturation point; for example, with a mixture of serum and $\frac{N}{35}$ acid there would be a faint purple tinge to the drop on the litmus, while with $\frac{N}{40}$ acid the drop would be blue. Having determined a fixed point in this gradual change of color from red to blue as our end reaction, we made it a rule to repeat each titration one or more times, and it was here that the shortcomings of the method were most apparent in the non-concordance of the results of repeated titrations of the same specimen at intervals of only a few minutes. Frequently the difference of reaction caused by a change from $\frac{N}{35}$ to $\frac{N}{40}$ or $\frac{N}{45}$ acid was so slight that a great element of imagination was necessarily introduced. If we express the terms $\frac{N}{35}$, $\frac{N}{40}$, and $\frac{N}{45}$ as actual sodium hydrate, this variation amounts to 14 to 22 per cent. The blood alkalinity in disease varies within narrow limits, and it is evident that a method with this amount of inherent error is hardly of sufficient accuracy to be of clinical value. As a matter of fact, in most of the diseases investigated, we have found the readings to fall within the limits of "normal variation" by this method.

When one attempts to explain the inaccuracy of this method, several factors must be considered. Is litmus paper a good indicator? While we shall not attempt in this note to discuss the nature of the chemistry of the blood, it is necessary to state that the blood is not a simple chemical solution, but consists of a solution of complex salts. It contains no free hydroxyl (OH) ions, but its alkaline reaction to litmus is probably due to basic carbonates and phosphates combined with protein molecules or weak acids. It must be remembered that although blood reacts alkaline to litmus, and more so to dimethyl-amido-azo-benzol, it is neutral or acid to phenolphthalein. The physiological activity of the cell protoplasm demands that the circulating nutrient fluid maintain a fairly constant balance, and the variation produced by disease, unless it cause the death of the cell, must always be within narrow limits. Hence, without considering the theoretical basis of indicator reactions, it is at once apparent that any indicator to be of value in hæmalkalimetry must be delicate and must show a definite end reaction. It is a well-known chemical fact that litmus does not show a sharp line of neutrality in the presence

of carbonates. It gives neutrality with carbonates at an intermediate point between the bicarbonate and the normal carbonate, and with phosphates at an intermediate point between the monobasic and the dibasic phosphates. Moreover, the inconstant factor of the viscosity of the blood influences the saturation point of litmus paper; for Hutchison (9) has shown that dilution of serum increases its permeability to litmus paper and thereby causes an apparent increase in its alkalinity as tested by the Haycraft-Williamson (10) method. Therefore, litmus paper is not a suitable indicator for such delicate titrations as are necessary in measuring the alkalinity of the blood. The inherent errors of the Wright method are probably mainly due to the insensitiveness of the indicator.

Furthermore, the mechanics of pipette titration, as employed in this method, would not seem to be satisfactory for even comparative chemical purposes. It is supposed that equal amounts of acid and alkali are used in each titration, but when one considers that the acid solution follows an alkaline fluid (serum) of greater and variable viscosity into a pipette of small caliber, it is obvious that identical amounts are not used, and that the error thus caused must be considerably magnified on account of the small amounts of material employed. If, however, as might be urged, this error should prove to be constant, it need not be considered in comparative work. We are at present investigating this point by using the pipette method with other indicators, but as yet our observations are not sufficiently numerous to justify an opinion as to how much of the error in the Wright method is due to the pipette.

To conclude, then, it is seen that in using the indicator and method of measurement proposed by Wright, the variation inherent to the method includes within its bounds the changes seen in those diseases in which the early recognition of acidosis is important. And when the lowered alkalinity falls definitely without the "normal," we have at the same time clinical and urinary changes which make the condition obvious.

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THE TREATMENT OF GONORRHŒAL ARTHRITIS BY VACCINES.

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In undertaking the study of the treatment of infections by means of vaccines, and especially the control of the administration of vaccines by means of the opsonic index, it has seemed advisable, in order to obtain any clinical evidence as to the value of this method of treatment, that we confine our attention as strictly as possible to one form of infection, or at least that we study as many cases of one condition as possible. We therefore began the study of cases of gonorrhœal arthritis, choosing this lesion for several reasons.

In the first place, it seems most likely that phagocytic or opsonic immunity may play a prominent part in the case of those infections due to the group of cocci to which the gonococcus belongs. Second, this is a condition in which treatment, as at present carried out, is not very satisfactory. Third, it is a local metastatic infection of prolonged course, situated in a position in which the blood supply is not very abundant, and in which, therefore, stimulation of the general mechanism of immunity is probably not very active. In the majority of cases it runs a chronic course, and therefore usually lasts a sufficient time for artificial immunizing processes, if of any value, to be effective. Theoretically, therefore, this would seem to be a most suitable condition for treatment by vaccines,

if the opsonic theory and method devised by Wright are correct.

Furthermore, it is a condition occurring with considerable frequency. The fact that during the past five months 20 patients with gonorrhœal arthritis, in whom the diagnosis was quite certain, applied to us for treatment, shows how frequently the lesion occurs, much more frequently undoubtedly than either the laity or most practitioners are aware, as many of these cases are undoubtedly wrongly diagnosed "rheumatism." It is also a condition which we have been studying for some time, and so were fairly familiar with the course under ordinary forms of treatment.

We have tried to enter upon this study with unbiased minds, though we have endeavored to exercise as much enthusiasm as possible, recognizing that upon this the results of any form of treatment are somewhat dependent. While the number of cases has not been very large, we have endeavored to study each one of them as carefully as possible, with the hope that we might obtain results which would be applicable to other analogous infections.

One difficulty in arriving at any conclusion as to the value of this method of treatment from the reports already made is that in most instances isolated cases of one condition have been reported. It is well recognized that we can never judge of the value of treatment from single cases, especially where the writer has apparently undertaken the treatment with great enthusiasm, owing to the attractiveness of the theoretical considerations upon which it is based. From some of the reports we might judge that the cures by means of vaccines have been almost miraculous, and undoubtedly much harm may be done to the general adoption of this form of treatment by these extravagant claims. From our observations, the value of this form of treatment must be estimated just as is that of any other form of treatment, and in the absence of any large statistics upon which final judgment must rest, we can only arrive at an opinion by as complete a study as possible of a single group of cases, the unfavorable as well as the favorable ones. We, therefore, feel justified in reporting in some detail the cases of gonorrhœal arthritis so far studied.

The technique employed in the estimation of the opsonic index in this series of cases was as nearly as possible that described by Wright. A few points of importance in connection with the work with the gonococcus, however, may be mentioned. The strains of gonococcus used in the estimation of the opsonic index were derived from two sources. One strain was isolated from a knee joint in a severe case of gonorrhœal arthitris with effusion (Case No. III), and the other from a case of gonorrhœal periostitis. Comparisons made between these two strains did not reveal any appreciable differences, and the indices obtained with each of them in the same case did not show any marked variation. But it may be advisable in spite of this fact, to use the organism isolated from the case under treatment. However, there are several difficulties here presented. In the first place, it is often a long and difficult task to isolate the gonococcus from a certain lesion. In the subacute and chronic cases this is especially true. Not infrequently it is quite impossible to find the organism, even in the genital lesions, and much more so in the joints of cases which are definitely gonococcal.

Blood agar was found to be the most satisfactory for the isolation and continued cultivation of the gonococcus. This was prepared by the usual method of mixing human blood with melted agar. About 0.5 cc. of fresh blood was mixed with 10 cc. of agar, and then the tubes were slanted in the usual way. The organism grew very readily on this medium and an abundant growth was obtained in 24 hours. Hydrocele agar was at first employed, but this was found unsatisfactory for several reasons. The growth on this medium is very scanty in 24 hours, and there is always a liability that the organism may die out unless transferred to a new tube daily. On the blood agar, however, the organism remains viable for several days. After repeated transfers are made during a period of several months, the gonococcus will then grow quite as readily on hydrocele agar as on the blood agar. The following points seem to be of interest in regard to the emulsion of gonococci to be used in carrying out the opsonin test.

The age of the culture is of extreme importance. At the most, it should not be over 24 hours, and preferably between 16 and 20 hours, as it was found that in older cultures numerous involution forms were present. Then the strength of the emulsion should be carefully estimated, which can only be done by experience. The most suitable strength is such that in normal serum an average of 6 to 10 cocci per leucocyte will be taken up. The mixing of the emulsion and the breaking up of clumps should be very thoroughly performed. This was accomplished by the method recommended by Wright. It was furthermore found that in the staining of the preparation more time was required than in making tests with the other pyogenic cocci. Hastings' modification of Romanowski's stain was used entirely. The diluted stain was allowed to remain on the slide for at least ten minutes, and more frequently up to half an hour. Even after this period some of the organisms were occasionally found to be very poorly stained, and others showed only as indistinct shadows. It might be mentioned here that similar results were observed when working with Diplococcus intracellularis of Weichselbaum. In counting the organisms, each individual coccus was taken as a unit and all diplococci were counted as two.

It has been held by some writers that certain strains of the organism are endowed with special powers in the production of opsonic immunity. Our experience has been quite to the contrary. The vaccines used by us were prepared from four different strains of gonococcus. Two of these strains have already been mentioned above. The other two were isolated from cases of acute gonorrhœal urethritis. In comparing the results obtained, no distinct difference could be demonstrated in the clinical results or effects on the opsonic index when the patient was vaccinated with a vaccine made of his or her own organism or when a different vaccine was used.

The vaccines were prepared after the method devised by Wright. The strength of each vaccine was usually 600 mil-

lion to one cubic centimeter. In carrying out the treatment, the opsonic index was first determined, and if below normal, the first dose of vaccine was given. This was usually 300 million gonococci. The following vaccinations increased gradually in amount, until 1000 million at one dose was used. No ill effects were encountered, even when 1200 million gonococci were administered at one dose. A slight local reaction always occurred 12 to 24 hours after the first dose. This consisted of slight pain, tenderness and redness at the site of injection. In only one case (No. 15) was this reaction at all marked. This patient suffered for 48 to 72 hours after the injection with marked pain and induration. This occurred after subsequent injections, as well as after the first one, but the reaction diminished in severity with each subsequent dose. After the last injection no reaction occurred.

General constitutional disturbances following the injections were very rare. In fact, in only one case can this be said to have occurred. In this case (No. 12) there was a sharp rise of temperature with general malaise the evening after the second injection. Recovery from this, however, was complete within 48 hours, and no further general disturbance was noted with the subsequent treatment.

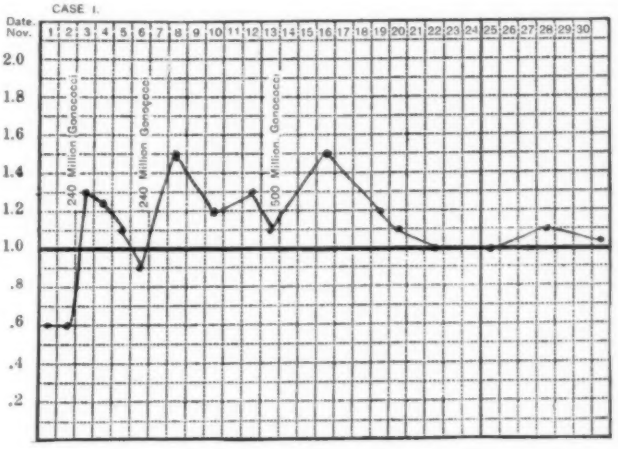
The change in the opsonic index after the treatment varied greatly in individual cases. For example, in comparing Cases 6 and 10, it will be seen they were both vaccinated with the same amounts on January 14, 1907. In Case No. 6 the index gradually ascended and reached the maximum on January 21, when it descended rather sharply. On the other hand, in Case 10, the ascent was sudden, reaching the highest point in two days, and then the index gradually fell. But in both cases the index reached normal in ten days. In studying the charts closely, it will be seen that the rule is a sudden ascent after the reaction, with gradual descent.

The number of vaccinations in the individual cases varied according to the severity and the chronicity. The smallest number was one and the largest eight. The intervals between the injections were controlled by the opsonic index, as stated above, though in most cases the intervals were from seven to ten days. A word might here be said concerning the "negative phase." In none of the cases has this been at all marked. It is possible that the absence of "negative phase" may be due to the fact that daily observations following the vaccinations were not made. But the fact remains that the ill effects of a "negative phase" were not observed clinically and a cumulative "negative phase" was never observed.

The following are reports of 15 cases in which each case has been followed during the entire course. Five other cases have been under observation for short periods, but for some reason it was impossible to follow them longer, mainly because the patients refused to carry out the treatment.

CASE I.—W. J. Dispensary No. 53,765, male, aged 25.

The patient came under observation November 1, 1906. He then complained of pain and tenderness in the right knee. He had had one attack of gonorrhœa several years before, unassociated with arthritis, and a second attack beginning September 3, for which he applied for treatment in the dispensary. On September 10 he began to have pain and swelling of the right knee. This continued and the swelling increased up to the time when seen by us. At no time were any other joints involved. The circumference of the right knee was found to be 8.5 cm. larger than the left. The patella was floating. The knee was held stiffly, there was marked tenderness and the local temperature was elevated. The patient was walking on crutches. He was allowed to continue going about and no other treatment was instituted. The treatment of the urethritis, however, was continued. Following the first injection there was marked improvement in the condition of the knee. Two days after the first injection the circumference of the joint had diminished 2.5 cm. The improvement continued uninterruptedly. By November 10 the pain and tenderness had almost entirely subsided, motion in the joint was good and he walked without a cane. He returned to work on November 19, all signs of joint involvement having disappeared. The urethritis subsided and a slight enlargement of the prostate was the only sign of genital infection remaining. There has been no return of the trouble since.



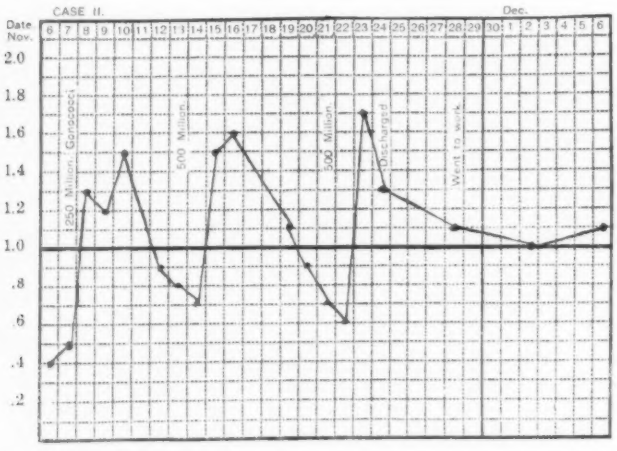
CASE II.—J. O. Surgical No. 19,743, male, aged 40.

The patient came to the hospital for treatment on September 26, 1906, complaining of pain, stiffness, and swelling of the right elbow joint. He had had a fracture of the lower end of the left humerus in December, 1904, from which he never completely recovered. On September 16, 1906, he contracted gonorrhœa for the first time. About one week after the onset of the urethritis he developed some pain and swelling in both wrists. This, however, did not trouble him much and soon subsided, but during the night of September 25, 1906, he began to have severe pain in the right elbow joint. Swelling, pain, and tenderness increased very rapidly and the patient came to the hospital for treatment. On examination the right elbow joint was found to be very much swollen, very tender, the motion limited to a marked degree and the surface temperature elevated. He had an acute urethritis of mild severity.

The joint was treated for several week by local hyperæmia, with only slight improvement. The urethritis was treated by injections and the discharge soon disappeared.

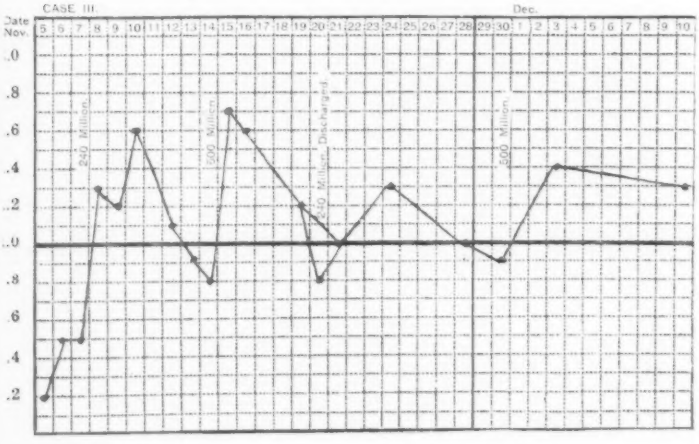
On November 7, 1906, the patient was vaccinated with 250,000,000 dead gonococci. There was immediate improvement. This improvement progressed steadily, with diminution of the swelling and tenderness. Motion in the joint became freer and he was discharged on November 24, and returned to work on November 28. At the time of leaving the hospital, a distinct crepitus could be elicited in the joint, but he did not feel any pain or inconvenience from this, and the patient thought that the motion in the joint was as good as it had been before the infection.

On December 13 the patient returned to the hospital suffering from acute lobar pneumonia. There had been no recurrence of the joint trouble. His pulmonary condition became very grave and he died on December 19. A few hours before death he



complained of a sharp pain in the right elbow, but nothing was made out on local examination.

At the post-mortem examination the right elbow joint was opened. The articular surfaces were roughened and showed signs of a recent severe inflammation. There was a slight amount of fluid present. Cultures from this exudate gave a pure growth of gonococci. Whether the gonococci remained latent in the joint and with a lowering of resistance again became active, of course could not be determined. This case is of great interest, however, showing the persistence of organisms in the joint, even though all signs and symptoms had disappeared.



Case III.—F. J. Medical No. 20,165, female, aged 26.

The patient had never previously had arthritis. During the spring and summer of 1906 she was troubled with leucorrhœa, pain, and frequency of urination, which a physician treated by injections. On September 10, 1906, she had severe chills and fever, and the following day had pains in all her large joints. She entered the hospital on September 18, 1906, complaining of painful and swollen joints. On examination there was pain, tenderness, and increased heat over the left knee, right wrist, and both ankles. Pelvic examination was negative. Although the right wrist and left ankle improved, the fever continued high and irregular and she became very anæmic. Cultures made from the right knee on October 8 were sterile. The fluid was purulent, and smears showed many polymorphonuclear leucocytes, but no organisms.

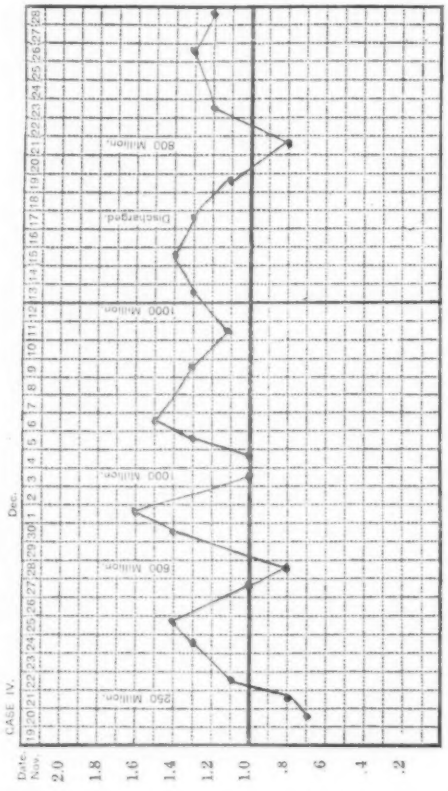
On October 13, cultures were made from the left knee and right ankle and from the blood. On the same date the knee and ankle were opened surgically and thoroughly irrigated. Cultures were again taken from the joints. No growth was obtained from the ankle or blood, but gonococci were grown in pure culture from the knee, both before and at operation.

The incisions healed very well, but the joints remained stiff and painful and the patient's general condition did not greatly improve. The opsonic index was frequently estimated and was found to range between 0.2 and 0.5. On November 7, the first dose of vaccine was administered. The condition improved rapidly and she was discharged on November 20, complaining only of slight stiffness in the left knee. This soon disappeared and all the joints were normal on November 28, 1906. Since then there has been no recurrence.

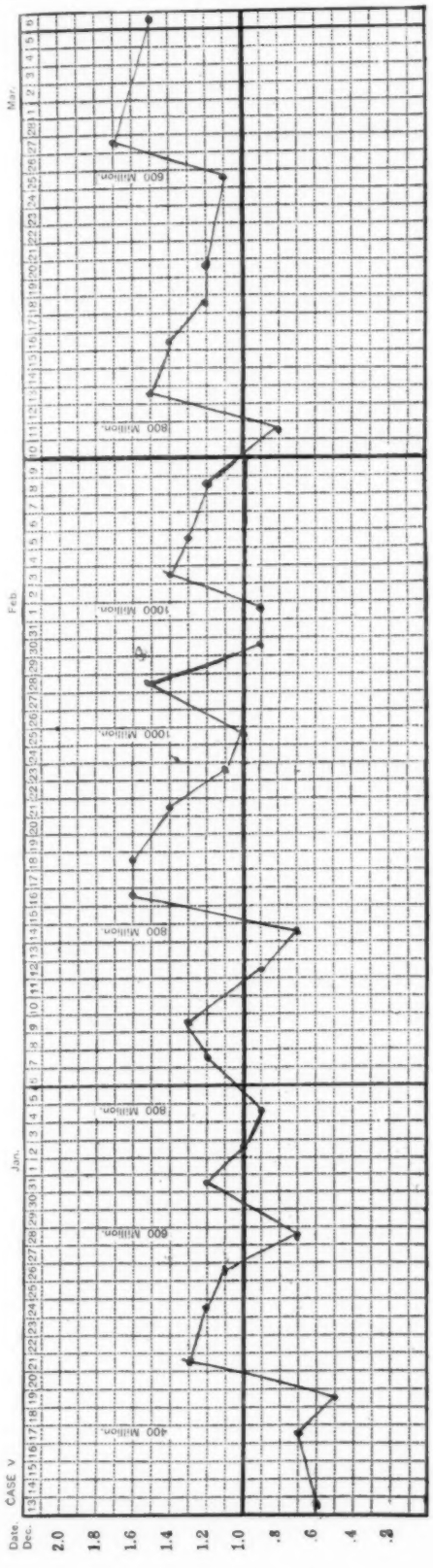
The evidence in this case seems fairly good that the vaccines helped, as improvement was progressing slowly when the vaccines were begun, and afterwards went on very rapidly to complete recovery.

Case IV.—M. S. Gynæcological No. 13,297, female, aged 20.

On October 13, 1906, the patient was operated upon for gonorrhœal pelvic inflammatory disease. Hysterectomy, double salpingo-oophorectomy and appendectomy were performed. The



patient made an uninterrupted recovery from the operation. On November 8 she began to have pain in the left elbow. This was followed by great tenderness, swelling, increased local temperature and fixation of the joint. The joint was treated with rest and local application of ice. But there was no improvement up to November 20, when the pain became so severe as to keep her



from sleeping, and she would not allow the joint to be touched. The opsonic index was 0.7. On November 21, all other treatment was discontinued and 250,000,000 dead gonococci were administered subcutaneously. During the following week the joint improved very much. The patient was out of bed on December 3 and was walking on December 5. During this time, however, she would not allow the joint to be moved and held it in a semi-flexed position. As a consequence, the joint became partially ankylosed, although all other signs and symptoms had disappeared by December 7. The patient was discharged on December 17, and failed to return for further treatment. In January she entered another hospital and was given ether and the joint forcibly manipulated. After this operation, the joint could be freely moved, although slight crepitus was evident. At the present time the patient has full use of her joint and there is no limitation of movement.

CASE V.—D. G. Dispensary No. 81,474, C. male, aged 26.

This patient had gonorrhœa in 1903 and again in August, 1905. Shortly following this last attack he developed arthritis in the knees and joints of the hands. He entered the hospital and was treated with hot air, with slight temporary improvement. He continued to have fresh attacks of arthritis and acute exacerbations of the urethritis. During December, 1905, and January, 1906, he developed prostatitis, double epididymo-orchitis, and vesiculitis. These complications, however, by proper treatment were soon relieved, with the exception of the prostatitis. Up to November, 1906, the arthritis was practically continuous, involving the knees, fingers, hands, sterno-clavicular joints, shoulders, hips, and ankles.

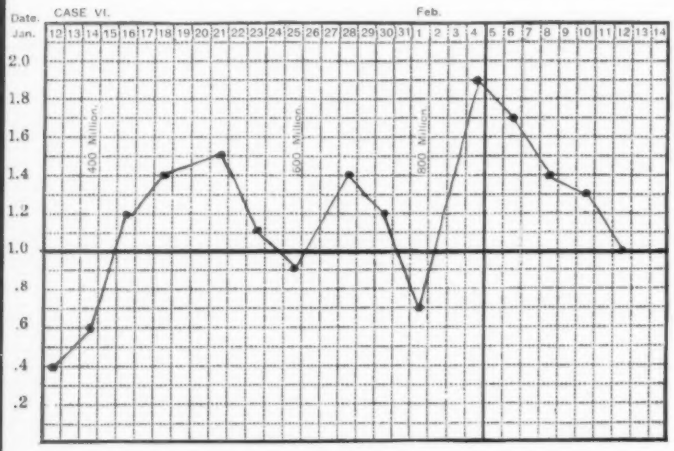
He first came under our observation on December 13, 1906. At this time he walked with a cane. There was pain and tenderness in the shoulders, hips, and sacro-iliac joints, with some diminished mobility. The opsonic index was 0.6.

On December 17, 1906, he was vaccinated with 400,000,000 dead gonococci. The improvement was slow, but quite marked. Within a week he walked without a cane. By January 12, only slight pain in the left shoulder and right hip remained, but he complained of pain on the bottom of the left heel. By January 21 these pains had all disappeared. The genito-urinary condition did not show a corresponding improvement. A chronic urethritis persisted and is still present. The prostate has shown little or no improvement. Since January 21 he has complained of dull pains in the extremities, not well localized in the joints. The patient is very neurotic and it is difficult to tell just how much pain he is having. There is no limitation of motion in any joint. He has complained of pain on the plantar surface of the left heel, but X-rays have shown no thickening or exostosis. It is possible that some slight localized joint or bone involvement still remains, though it cannot be discovered. He still complains of pain, however, and claims that he is unable to go to work. This case is the most resistant one we have had and has offered a good example of the neurotic tendency following gonorrhœal arthritis. The patient has persisted in having intercourse during the treatment and has undoubtedly been reinfected. This case shows the slight value which vaccines may have in the treatment of acute and subacute urethral affections.

CASE VI.—S. W. Surgical No. 20,168, female, aged 46.

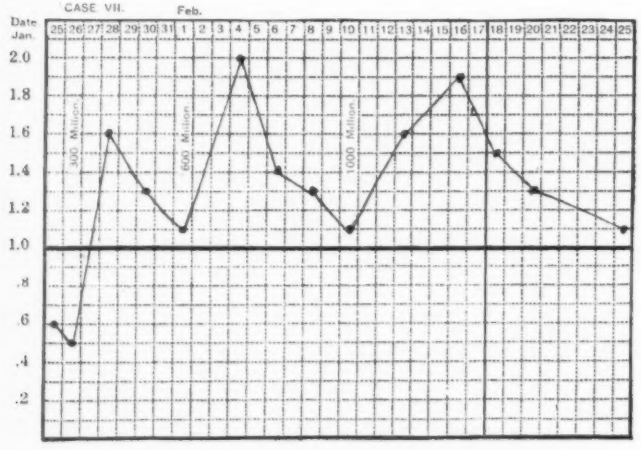
On December 14, 1906, the patient developed pain in the right hip. During the same day this disappeared, but the right shoulder became involved. This joint was very painful, and she could not use it. She came to the hospital on January 7, 1907. There was no history of gonorrhœa or acute articular rheumatism. The right shoulder was enlarged, tender, very painful on movement and there was increased local temperature. Smears taken from the vagina showed typical gonococci. White blood

cells numbered 9040. The opsonic index was 0.5 to gonococcus. On January 14, 400,000,000 dead gonococci were injected subcutaneously. There was immediate improvement and the patient was discharged on January 28, 1907, and returned to work on February 4, with perfect motion restored and no pain. There has been no return of the symptoms since.



Case VII.—H. K. Dispensary No. 11,266, D., male, aged 30.

The patient has had four attacks of gonorrhœa. The last was in October, 1906. Shortly after this he developed pain in the thyroid cartilage, which lasted for some weeks. In November, 1906, he had pain in the lower dorsal vertebræ. There was no local sign besides tenderness and pain on movement. In December the sacro-iliac joints became painful and at the same time the right ankle was very much swollen and tender. He continued in about this condition until first seen by us on January 25, 1907. At this time the right ankle was swollen, red, tender, and the surface temperature was elevated. The joints of the first and sec-



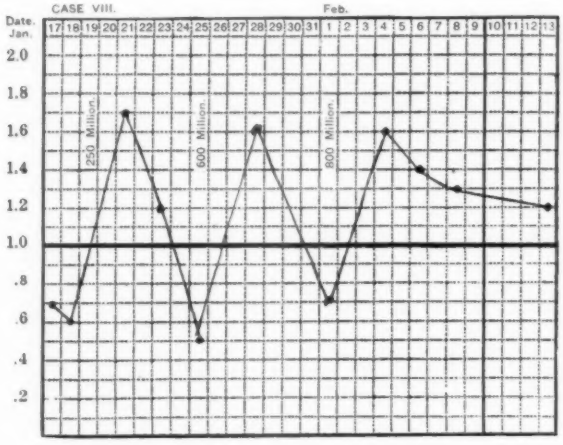
ond toes of the left foot and the little finger of the right hand were in a similar condition, while the sacro-iliac joints were painful and tender. He was compelled to use two canes. His opsonic index was 0.6 to gonococcus. An injection of 300,000,000 dead gonococci was immediately given. The patient began at once to show slight improvement. On February 7, all the joints had cleared up, except the sacro-iliac joints and the right little finger, and he had dispensed with his canes. Very soon the signs in those joints also disappeared, and but a slight capsular thickening of the middle joint of the right little finger remained. All objective signs in the sacro-iliac joints disappeared, but he still has a little pain over the sacrum in the morning on arising. He returned to work on February 16.

The genito-urinary examination when first seen by us revealed a chronic urethritis and prostatitis. This was treated by prostatic massage, during the time of vaccination. This improvement in the prostatic condition was very slow and the vaccination seemed to have very little effect on it.

Case VIII. C. S., male, aged 19.

The patient contracted his first attack of gonorrhœa in November, 1906. This was followed in a few weeks by pain, swelling, tenderness, and redness with increased temperature of the left wrist and the right ankle, while many other joints in the body were attacked very slightly in a fugaceous manner. He was treated in another hospital for some weeks with little or no improvement.

He was first seen by us on January 17, 1907, when the left wrist and the right ankle were much swollen, tender, reddened and there was increased local temperature. His other joints were painful at times, without objective signs. His opsonic index to



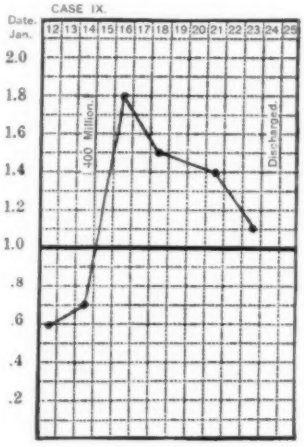
gonococcus was 0.6. On January 19, he received his first vaccination of 250,000,000 dead gonococci. There was a gradual improvement at first. He felt very much better in general health, although the joints did not show much improvement until after the second vaccination on January 25. Then the improvement became very marked and he returned to work on February 8, 1907. There has been no recurrence since.

Case IX.—C. A., Medical No. 20,608, male, aged 36.

The patient had gonorrhœa four times, the last attack in March, 1906. He never had rheumatism before. On December 29 he began to have pain in the lumbo-sacral region of the back, and in the right hip. This was followed by swelling, pain, and stiffness in both ankles and knees, which confined him to a chair until January 7, 1907, when he entered the hospital.

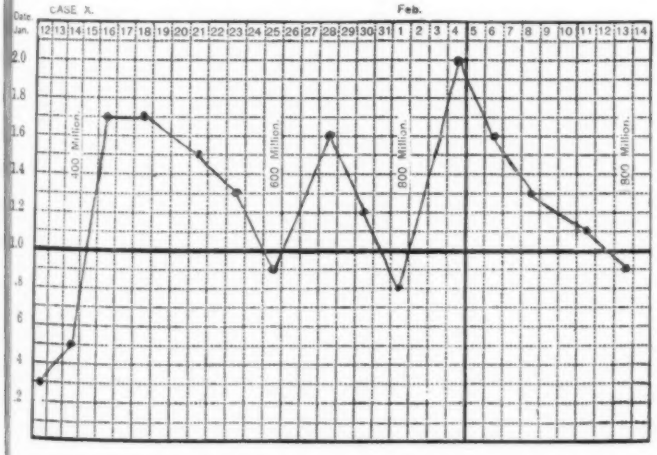
On examination the right knee and ankle were very much swollen, tender, and red and there was increased surface temperature. Genito-urinary examinations showed chronic urethritis and prostatitis. The opsonic index was 0.6 to gonococcus. A subcutaneous injection of 400,000,000 dead gonococci was given. There was some improvement and the patient was discharged on January 24, 1907. The improvement continued and there has been

no return of the arthritis. On the other hand, the genito-urinary condition has shown little or no improvement.



CASE X.—O. K., male, aged 26.

In November, 1906, the patient contracted his first attack of acute gonorrhœa. This was followed in a few days by pains in all his joints. At first there was no special localization, but it soon settled in the right knee joint. This became very much swollen, painful, exquisitely tender, and movement was much limited. He had a temperature of 103 degrees Fahrenheit, and there was a local elevation of temperature over the joint. His condition became progressively worse. The temperature ranged between 100° and 103° F. The joint continued to enlarge and there was fluctuation present. This was practically the condition of the patient when first seen by us on January 12, when the opsonic index to gonococci was 0.4. He was given his first vaccination on January 14, 1907. This was followed by immediate, although slight, improvement. The temperature became normal and remained so after 48 hours. The tenderness and pain subsided and the patient was out of bed on January 25 and was walking about with a cane on January 29. There remained some swelling and stiffness of the joint, which gradually disappeared and the joint regained its former condition, except for some thickening of the capsule. The urethritis had practically disappeared before vaccination was commenced.

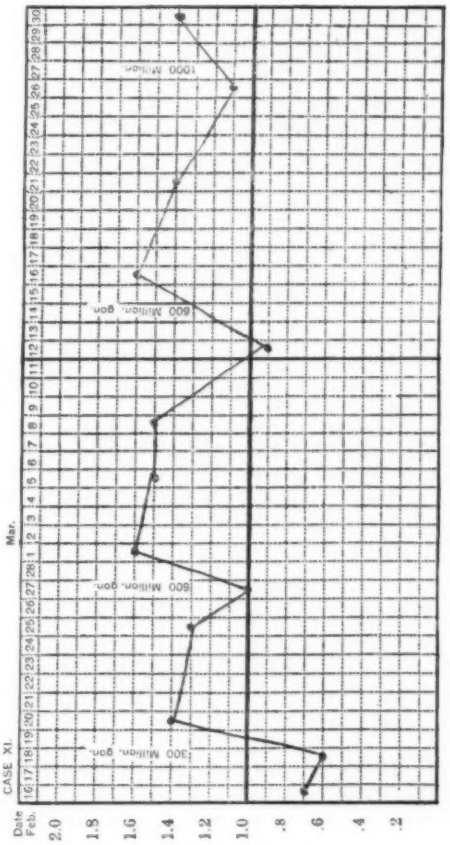


CASE XI.—N. B. Hospital No. 57,936, male, aged 35.

The patient has had gonorrhœa six times. The last occasion was in November, 1906. About December 1 he began to have pain in his hip and this soon involved his other joints in a mild degree. The right ankle grew worse and he entered the hospital on February 1, 1907.

On examination the patient was found to have swelling, tenderness, and slightly increased temperature about the right ankle. There was some morning urethral discharge, chronic urethritis, and prostatitis.

Treatment by Bier's hyperæmia method was begun, but the patient showed no improvement. At the same time urethral irrigations and prostatic massage were instituted. On February 16, 1907, the opsonic index was found to be 0.7 and on the 18th 0.6. At this time the former treatment was discontinued and vaccination begun, with a first injection of 300,000,000 dead gonococci. The improvement, although slow, was continuous. The ankle joint soon became normal, but the prostatitis was more resistant and patient was discharged on March 30, 1907.

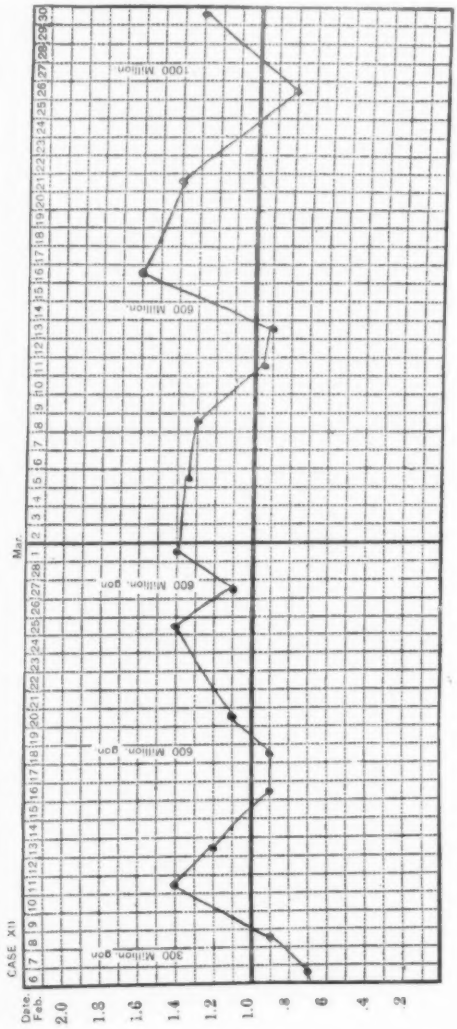


CASE XII.—C. B. Hospital No. 57,952, male, aged 35.

The patient contracted gonorrhœa on November 16, 1906. This gave him very little immediate trouble, but about January 15, 1907, he began to have pain in the lumbo-sacral region and both hips. The knees and ankles became swollen and tender and the patient entered the hospital on February 3. On admission there was a great deal of pain and tenderness over the sacro-iliac joints and in both hips, especially the right. The left knee was swollen and tender, and the patella was floating. The left ankle was much swollen, hot, tender, and red. On genito-urinary examination there was found to be chronic urethritis and prostatitis. The opsonic index was 0.7 to gonococcus. The sacro-iliac and hip joints were treated by the application of a plaster jacket, which

the patient promptly took off. The other joints were treated with hot fomentations, and the genital condition by irrigation.

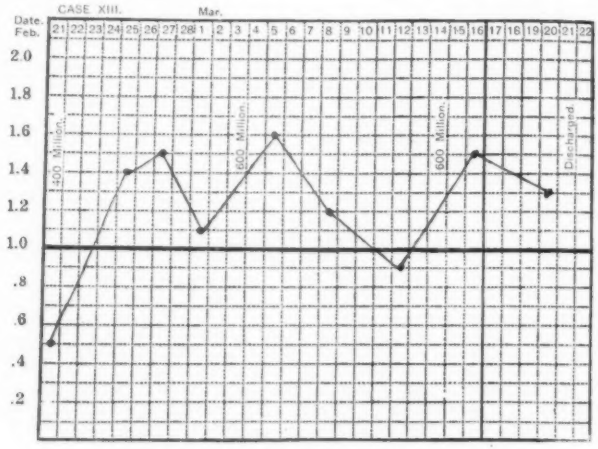
On February 7, 1907, the first vaccination of 300,000,000 dead gonococci was given. There was slight but immediate improvement. By February 21, the ankles and knee had returned to a normal condition. The sacro-iliac and left hip joint showed very slow progress, although the patient was up in the chair on February 22, and even in these joints there was, however, distinct improvement, and the patient was discharged on March 30, as he refused further treatment. The genito-urinary condition had shown steady improvement.



Case XIII.—D. M. Medical No. 20,780, male, aged 32.

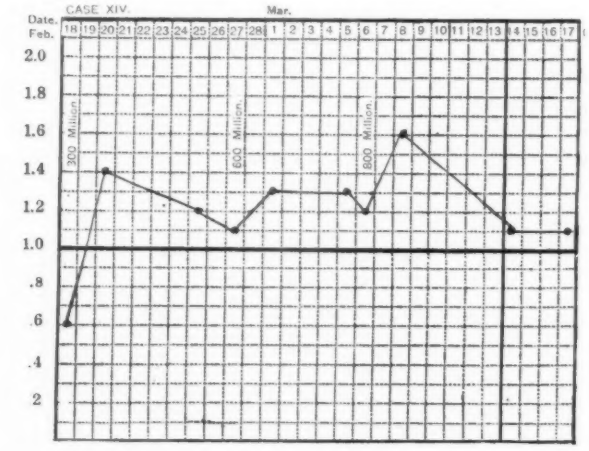
In the early part of December, 1906, the patient contracted his first attack of gonorrhœa. This was followed on December 28, by sudden pain in the left wrist. This became swollen and reddened. Later the middle finger of the right hand was involved and he had transient pains in all his joints. He was not confined to bed, but was compelled to give up his work. There was no improvement, and the patient entered the hospital on February 19, 1907.

On admission the left wrist and hand were very much swollen, reddened, painful, and tender, with increased local temperature. Voluntary movement was absent in the wrist and fingers, while passive movement was very painful. The patient was vaccinated on February 21, with 400,000,000 dead gonococci. There was apparently immediate improvement. Within a few days the redness and pain had disappeared and the swelling and tenderness were much diminished. He was soon able to move his fingers voluntarily to a slight extent. All the signs gradually subsided except the limitation of movement, which persisted to some degree, when the patient was discharged on March 21, 1907. Since then the joint has practically regained its normal condition.



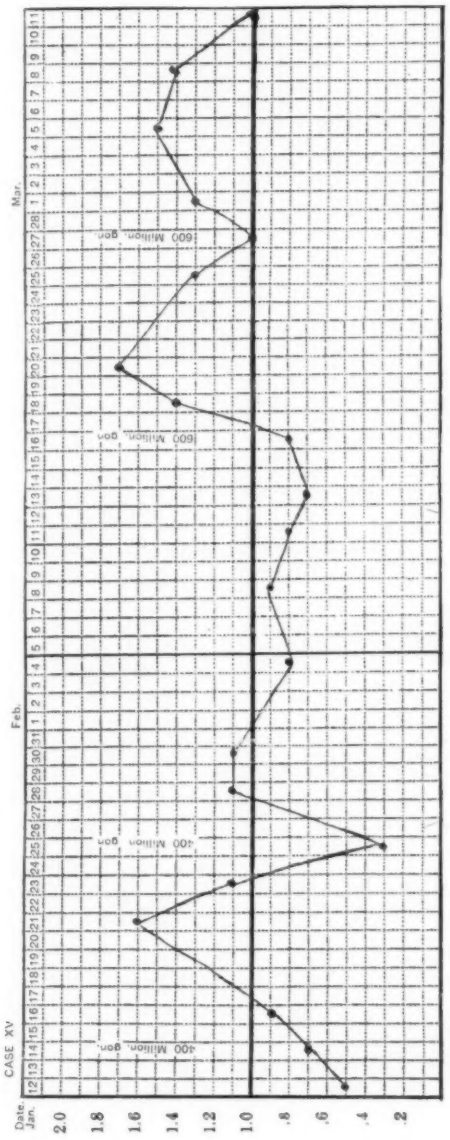
Case XIV.—A. D. Surgical No. 20,209, female, aged 32.

For the past two years the patient has suffered from a persistent leucorrhœa. On January 24, 1907, she began to have pain in the right ankle. This increased very rapidly until the pain was intense. The whole foot became swollen to a marked degree and she had a temperature of 103° F. The patient was admitted to the hospital on February 12. On examination the right foot and ankle, which were the only joints involved, were very much swollen, very painful and exquisitely tender. There was redness and increased local temperature. The motion was



very limited and the patient was unable to walk. Gonococci were not demonstrable in the vaginal discharge. On February 18, 1907, the opsonic index was 0.65 to gonococcus and she was then vaccinated. The improvement was immediate. The local signs of inflammation soon subsided and patient was walking on March 5, and was discharged on March 8. At this

time there were no local signs, but on walking there was some pain in the right instep. Before the onset of the arthritis there had been very marked flat feet. A support was given the patient for the right foot, which gave great relief, and there has been no recurrence of the inflammatory condition.



CASE XV.—J. P. Male, aged 41.

When the patient was 20 years of age he had an attack of gonorrhœa, which lasted ten days and was uncomplicated. He did not have any return of the symptoms until June 1, 1906. At this time he developed an acute urethritis, the discharge from which contained gonococci. In July, he suddenly began to have pain in the left knee joint. This became very much swollen and tender and there was a good deal of redness. During the summer the pain and redness subsided, but the swelling remained. In September a plaster cast was put on, keeping the knee in an extended position. This was removed in October, as the leg had become very much swollen and there was a good deal of pain present. The œdema of the leg disappeared, leaving the knee still swollen and painful. It remained in this condition until seen by us on January 12, 1907.

Examination at this time showed the left knee joint to be very much swollen, circumference 44 cm., that of right knee joint 35 cm. There was tenderness and marked limitation of movement. Genito-urinary examination showed a chronic urethritis, with a morning discharge, and a chronic prostatitis. The opsonic index to gonococcus was 0.55. On January 14 he was given 400,000,000 dead gonococci subcutaneously. The pain in the joint disappeared within a few days. The urethral discharge, however, became more profuse at first, but after seven days it ceased altogether. The swelling slowly subsided and the motion improved, but the treatment was discontinued from January 29 until February 15, as the patient was suffering from influenza.

On February 16 the circumference of the left knee was 38½ cm. and the motion was slowly improving. By February 27 there were no signs of inflammation and massage was advised. This, however, caused a good deal of pain over the anterior border of the tibia and had to be discontinued. By March 5, the right knee joint had diminished to 37 cm. and the movement was gradually improving. Since that time this has steadily increased and there has been no return of the acute symptoms. At present the knee can be bent almost to a right angle and the patient is going about very much pleased with the result. The prostatitis, however, has shown very little improvement since the treatment was commenced, although prostatic massage has been carried on concurrently.

This is the only case where local reaction to any extent was encountered at the point of vaccination. After the first two vaccinations there was marked tenderness, redness and induration about the site of injection. This, however, did not extend further, and after the last two vaccinations the effect was nil.

CHART OF CASES.

No.	Name.	Age.	Sex.	Duration of illness before vaccination.	No. of vaccinations.	Average interval.	No. of days under treatment.	No. of days before returning to work.	Range of opsonic index.
						Days	*Days*	*Days*	
1	W. J.	25	Male	8 weeks	3	6	18	18	0.05 to 1.45
2	J. O.	40	"	7 "	3	7	17	21	0.6 " 1.55
3	F. J.	26	Female	8 "	4	8	23	23	0.2 " 1.6
4	M. S.	20	"	2 "	5	8	36	..	0.7 " 1.6
5	D. G.	26	Male	18 mos.	8	9	70	30	0.5 " 1.7
6	S. W.	46	Female	31 days	3	9	21	21	0.5 " 1.9
7	H. R.	30	Male	11 weeks	4	10	29	29	0.6 " 1.95
8	G. S.	19	"	8 "	3	9	12	20	0.5 " 1.65
9	C. A.	36	"	17 days	1	..	9	9	0.6 " 1.8
10	O. K.	28	"	7 weeks	4	10	30	..	0.4 " 2.0
11	N. B.	35	"	9 "	4	12	37	37	0.6 " 1.6
12	C. B.	35	"	4 "	5	12	48	..	0.7 " 1.6
13	D. M.	41	"	8 "	3	11	27	27	0.5 " 1.6
14	A. D.	32	Female	4 "	3	8	18	21	0.6 " 1.6
15	J. P.	41	Male	7 mos.	4	15	44	30	0.3 " 1.7

Many interesting points have come up in the study of these cases into which we cannot enter here. As to the question of diagnosis, we may say that in practically all cases this has been quite definite from the clinical course, the association of a genital infection or the isolation of the gonococcus. In Case XIV the proof of the gonococcal nature is perhaps not very convincing, but the clinical course rendered it quite probable.

Having followed these cases from day to day, it is difficult not to feel that the treatment has been of value. We recognize, however, how difficult such conclusions are, and we pre-

fer therefore to publish the cases as they are and allow others to decide whether they will carry out this form of treatment or not.

We realize that many cases of gonorrhœal arthritis recover completely under other forms of treatment, but it must be remembered that persisting ankylosis is not at all infrequent. The results in those cases which had been treated by other methods were quite marked. In Case III, although operation had been performed, the progress was very slow until vaccination was commenced. The results in the chronic cases were more marked than those in the acute ones. Case XV was of especial interest on account of the long duration preceding treatment, and the very favorable result that was obtained.

On the whole, our impression is that the vaccine treatment as carried out, has been of distinct value, though we realize that it would require many more cases to make this conclusive. They have been sufficient, however, to justify us in continuing. Those who expect brilliant results or immediate cures following one or two doses of vaccine will be disappointed. We have omitted as far as possible all other forms of treatment, as we have wished to test the vaccine alone. There is no reason, however, why they should not be combined with whatever form of treatment may be considered advisable.

As to the value of the opsonic index in diagnosis and as a guide to the administration of vaccines, we are more undecided. The estimation has been carried out with as careful technique as possible—counting, however, only 50 cells. During the progress of the study, however, work carried on in this laboratory by Dr. Moss, and in which the writers have shared, seems to show that very considerable fallacies exist in our present method of estimating the opsonic content of the blood, and while we are not yet able to say exactly what these limits of error are, they seem to be sufficient to render of little value even considerable variation in the index. It will be seen, however, from a reference to the charts that they resemble quite closely those made by Wright and other observers. In most cases the index has risen following the injection, though at varying intervals. But it must be borne in mind that as the injections were usually made when the index was low, in an irregular chart due to accidental causes, the chances would be greater for the curve to go upward. It is of considerable interest that the first index in practically all of our cases was below 0.7 and in a number of cases 0.5, in one being only 0.2. It is quite probable that such low indices are not due to variations in technique, but are due to real diminution in the opsonic content of the blood. We do not feel, however, that even this can be absolutely decided at present. A very low index may speak for a gonococcus infection.

A reference to our charts will show that the vaccines were usually given at intervals of from 7 to 10 days. Considering the present almost certain inaccuracy in opsonic technique, and the absence of sufficient evidence as to the rôle of opsonins in immunity, it hardly seems advisable that the control of the administration of vaccines by the estimation of the opsonic index should be persisted in. We do not feel that the danger of cumulative negative phases is a real one. In no case have we seen the administration of gonococcus vaccine do harm, and we feel that these cases offer sufficient justification for the treatment of gonorrhœal arthritis by means of vaccines in doses of 500 to 1000 million, administered every 7 to 10 days. Statistics, however, from a large number of cases treated in this way will furnish evidence for or against its value.

THE TUBERCULO-OPSONIC INDEX AND TREATMENT BY TUBERCULIN.

By P. C. Jeans and A. W. Sellards.

(From the Biological Division of the Clinical Laboratory.)

As all who have worked with the tuberculo-opsonic index have found the greatest difficulty in the preparation of a proper emulsion, we have spent considerable time preparing emulsions according to the various methods advised by various observers, in the attempt to procure a perfectly homogeneous suspension. The sources of the tubercle bacilli with which we have worked were: (a) crumbs obtained from Dr. Baldwin, of the Saranac Laboratory, which were secured by filtering a broth culture of the tubercle bacillus, and (b) a glycerine agar-agar culture obtained from Dr. Ford, of the Bacteriological Laboratory of the Johns Hopkins Hospital, and said to be directly descended on artificial cultivation, from an original culture obtained from Koch many years ago.

In the preparation of the emulsion according to the directions of Wright and Douglass,[1] the bacteria should first be heated to 100° C. to destroy their power of clumping, and then ground in an agate mortar with 0.1 per cent salt solution, any stronger solution inducing spontaneous clumping. The remaining clumps are then removed by centrifugalization. Later Wright advised the use of 1.5 per cent salt solution to avoid spontaneous phagocytosis. As the heating of the bacteria causes extreme clumping, the grinding becomes a tedious and time-consuming process, but gives a homogenous emulsion containing a few clumps, many isolated bacteria, some dust particles and many fragmented cells. Considerable difficulty is encountered in enumerating these fractions of cells in phagocytic preparations. We find that some observers have counted each fragment as one bacillus; others have attempted to estimate the fractional parts of bacilli. Either method offers considerable chance for error. In our experience we found that we could handle smaller quantities of bacilli and with

[1] Proc. Roy. Soc., Lond., 1904, LXXIV, 161.

less danger of contamination when we substituted for any of the shaking devices or the agate mortar a test-tube 5 cm. long and 5 mm. in diameter, made from heavy glass tubing. We made a pestle from a glass rod 3 mm. in diameter. The avoidance of contamination becomes of paramount importance, since, after the emulsion is once obtained, it will be clumped again if heated for sterilization.

The only method by which we have been able to separate the bacilli without causing fragmentation is to emulsify the living bacilli, thus avoiding the extreme clumping caused by heat. The emulsion is prepared in the same way as for a living staphylococcus emulsion by rubbing up the bacilli in a little salt solution with a heavy platinum wire or small glass rod. In order to dispense with the precautions necessary in working with a living emulsion we sought a means of sterilizing the emulsion without causing clumping. According to Kolle and Wassermann[2] tubercle bacilli are killed in a few hours by direct sunlight. We have found that an emulsion in salt solution when exposed to the direct sunlight is killed in at least ten hours. The emulsion, prepared in this manner, has the following advantages:

1. The separation of the bacteria is effected without fragmentation and with scarcely more work than is required for a staphylococcus preparation.

2. Sterilization is effected without clumping.

3. When the concentration is sufficient to give a count of 5-8 bacteria per leucocyte, the cloudiness is scarcely discernible to the naked eye, in contrast to the milky emulsions usually employed.

After our emulsions were once obtained by either of the above methods, spontaneous clumping has not been a troublesome factor in either 0.1 or 1.5 per cent salt solution. We were not able to determine that the concentration of the salt solution between 0.1 and 1.5 per cent has any influence on spontaneous phagocytosis,[3] at least with the cultures employed by us.

We have always prepared a leucocytic emulsion from the leucocytic cream obtained by centrifugalization, attempting to secure uniform distribution of the leucocytes. We have collected our serum samples in the usual way, and have incubated our phagocytic preparations, generally for fifteen minutes, observing the usual precautions. We secured satisfactory graduation marks on our pipettes by a method suggested by Barber. A very delicate, almost capillary, glass hair is dipped into Brunswick black. On the capillary pipettes it makes a sharp, definite line, in contrast with the broad diffuse marks usually obtained with a blue grease pencil. The smears were made as advised by Wright.

We fixed our smears by covering with methyl alcohol for 30 seconds and stained with carbol-fuchsin for one to two hours at 37° C., decolorizing with 95 per cent alcohol three to four minutes, and counterstaining five to ten minutes with saturated aqueous methylene blue. Both hot carbol-fuchsin and the use of acid as decolorizer were avoided on account of the injury and distortion caused in the preparation, especially to the leucocytes.

Limits of Error.—As the variation of the count in individual leucocytes ordinarily varies markedly (even with low indices) the average count in 50 leucocytes is subject to considerable variation. In many preparations the leucocytes collecting along the edge of the smear contain distinctly more bacteria than those toward the center of the smear. Thus, by dividing a slide into three sections by transverse lines we found that the section at the end of the smear contained 282 bacteria, the middle section 107, and the first section 140 bacteria in 50 leucocytes. That the bacteria counted at the end of the smear were not mechanically superimposed was shown by control preparations with salt solution in which the leucocytes were everywhere free from bacteria. Ridges of leucocytes may occur almost anywhere toward the end of the smear, making it impractical to count control preparations a comparable distance from the end of the smear.

The following figures show the variation which may occur in the same slide and in duplicate preparations from the same serum sample. (a and a′ are counts on various parts of two slides made from duplicate preparations of the same tube of serum; b and b′ from duplicates of a serum sample collected from a case of tuberculous peritonitis with fever):

A.

	Bacteria in 50 leucocytes.			Total
a	282	107	143	
a′.....	288	235	204	1259
b	223	147	115	
b′.....	203	251	126	1065

B.

a	118	144	138	
a′.....	96	181	130	807
b	134	199	144	
b′.....	143	149	130	899

These counts were made after an experience of over six months, during which time a number of tuberculo-opsonic indices were estimated almost daily. Some differences are to be expected in the two series of counts, as no two men can employ exactly the same technique, but if the method is of value the relative counts should be the same. The opsonic indices obtained by each of the men, if we consider the total cells counted by each, do not show extreme variation. In one case 0.8 and in the other case 1.1. But when we come to consider the individual 50's of cells counted, the variation and inaccuracy appear. We may get indices in A's preparation from 0.4 to 2.34, or if we only take comparable parts of the slides, 0.56 to 2.34. In B's preparation we get variations on comparable parts of the slide from 0.82 to 1.49, or if any parts of slides be considered 0.71 to 2.07. If we add all the counts together we get $\frac{1964}{2066} = 0.95$.

It is quite evident from these figures that, so far as our counts go, the figures obtained from 50 leucocytes give us no

[2] Kolle and Wassermann: Handbuch der pathogenen Microorganismen. Jena, 1903. Bd. II.

[3] Proc. Roy. Soc., Lond., 1905, LXVII, 215.

idea as to the exact opsonic index. We may suppose that counts of 300 cells are sufficient, as the index obtained from these gives us about 1, or 0.95. But there is no evidence that this is correct. To find the absolute error in the technique it will be necessary by counting many thousands of cells, to find at what limit the error is negligible. Then having this we will have to count different preparations and again find the limits of error as the only errors do not occur in the counting. It is unnecessary to go into this, however. We have said enough to show that the limits of error, in our technique at least—and we have followed the method of Wright as closely as possible—are so great as to render the method inapplicable.

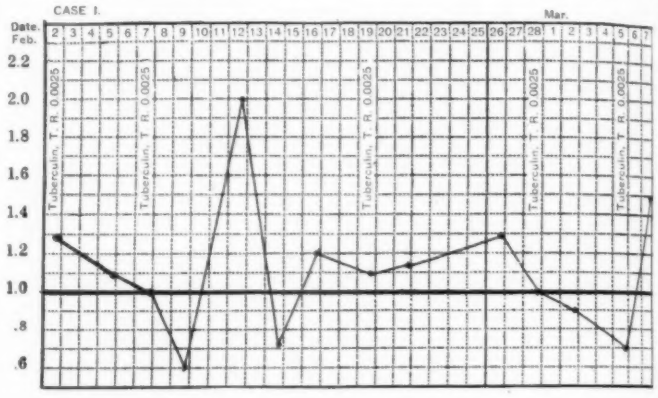
Probably large differences in indices mean differences in opsonic content, but at present we cannot feel that moderate differences mean anything. To count enough cells and to make enough controls of our preparation to render the work accurate places the method outside clinical application. Moreover the evidence that under normal conditions the opsonic content does not undergo considerable variations is not convincing.

But although we feel that our control by the opsonic index is untrustworthy, nevertheless the results obtained by the administration of TR. tuberculin in small, infrequent doses as Wright advises, have given encouraging results. It is possible that the method of vaccination is a correct one, even though the control of the administration by the opsonic index is inadequate. We, therefore, consider it important to briefly report our cases, together with a few opsonic charts which show that the fluctuation in the curves obtained by us do not differ greatly from those obtained by Wright and others.

Theoretically the most suitable cases for treatment with tuberculin would seem to be those cases of localized tuberculosis in which the presence of secondary infection is unlikely or very improbable. Such localizations are found in the glands, bones, and joints. In tuberculosis of the bladder and lupus the rôle of secondary infection cannot, of course, be excluded, but in the cases we have had under observation it has seemed to be of little importance. In pulmonary tuberculosis, however, secondary infection probably plays a considerable part, and it is therefore hardly fair to employ such cases to study the effect of any specific treatment. We, therefore, have confined our attention to the first mentioned group of cases. We have limited the number of cases, preferring to study the opsonic curves as accurately as possible. The following are abstracts of the cases which we have treated by this method. TR. tuberculin was used exclusively. In all cases the dosage was between 0.001 and 0.0025 mgs. In all cases frequent determinations of the opsonic index have been made and an attempt has been made to regulate the dosage and to interspace the administration upon the results of these determinations as advised by Wright. As a matter of fact, however, the doses have usually been given at intervals of from eight to ten days. The occurrence of negative phases has not been marked in any of our cases, and this phenomenon has played little rôle in the treatment. The five curves which we publish have been chosen from our more recent work, when our emulsion was most satisfactory, and after we had had considerable practice in the technique. It will be seen that the apparent regularity is only superficial, and that the variations can partly be accounted for by the laws of chance. Moreover the curve in the case of No. II, in which the result was very unsatisfactory, does not differ materially from that of the case No. I in which the results have been excellent.

CASE I.—*Tuberculous glands of neck; pulmonary involvement.*

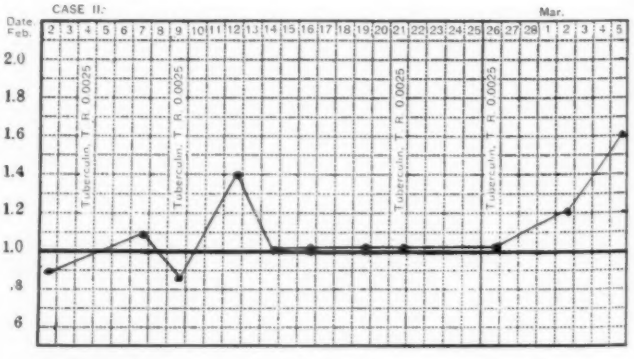
This patient, a young woman, had had a previous operation for enlarged glands on the right side of the neck, but with recurrence. When she came under our observation there were a few large



glands on the left side of her neck and a large group at the apex of the anterior triangle on the right side.

Physical examination of the chest showed signs of a slight tuberculous involvement of the right apex. After four months' treatment, during which time sixteen injections had been given, the glands were still enlarged, but reduced in size and decidedly less firm, and their size on measurement was just one-half what it was when the treatment was commenced. The râles in the chest had disappeared and the patient's general condition was excellent.

During these five months the patient remained out of doors almost constantly, sleeping out at night; she received good food and kept fairly quiet. A portion of the opsonic chart is shown.



CASE II.—*Tuberculous glands of neck.*

This patient had had tuberculous glands of the neck removed eight months previously, but the enlargement re-appeared. When she came under our observation the glands on the right side were markedly enlarged. There was no pulmonary involvement. During a period of two and a half months she received nine

injections of tuberculin. There was no decrease in the size of the glands to be made out. The patient thought they were slightly softer, though this was not definite. The patient, rather against our advice, decided to discontinue the tuberculin treatment, and to have the glands again removed by the surgeon. During the period of treatment the patient was employed by day in a telephone office. She refused to sleep out of doors at night, and the food could not be carefully regulated. A portion of the opsonic chart in this case is appended.

CASE III.—*Tuberculous arthritis of right hip and abscess of soft parts.*

The abscess was opened and drained, no attempt being made to reach the joint. The patient was subjected to the usual hygienic measures, good food and fresh air. Two months after the operation and while the wound was healing, the tuberculin treatment was begun. She received six injections in all, covering a period of one and one-half months, when she was discharged from the hospital, the abscess having entirely healed. During the stay in the hospital the power of flexion increased from 15° to 110°; possible rotation in and out increased from 10° each to normal; abduction increased from 5° to 45°.

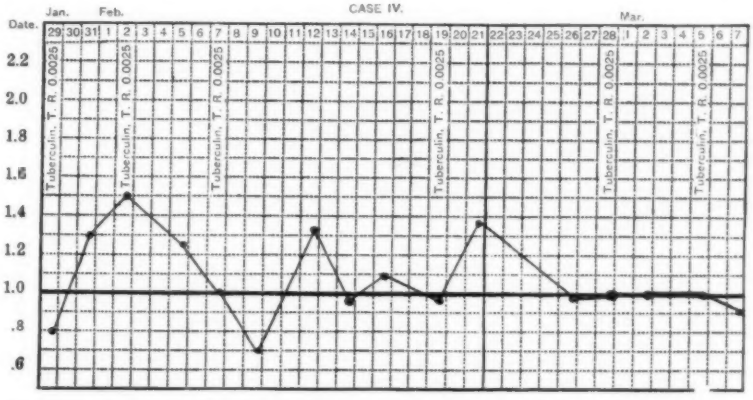
CASE IV.—*Tuberculosis of head of humerus.*

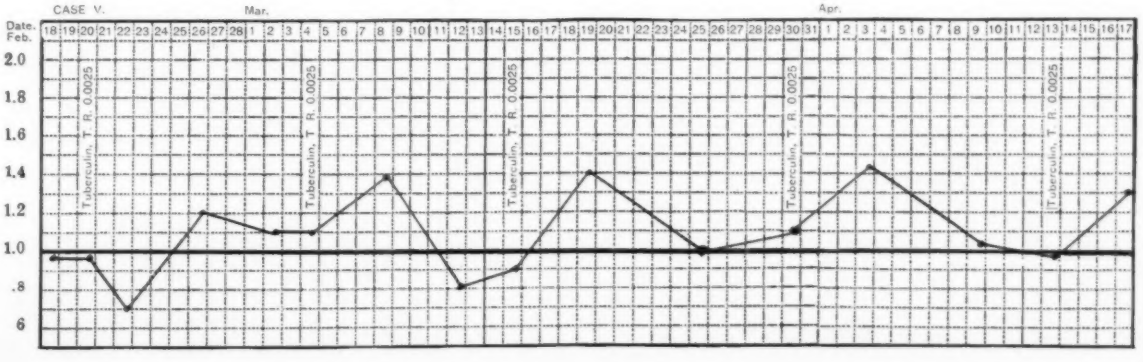
This patient, a boy eight years old, had had his shoulder injured thirteen months previously while at play, but the pain subsided in a week. Following this he had frequent attacks of pain in the shoulder with limitation of motion, these symptoms becoming more marked during the three weeks before coming under our observation. At this time there was found to be atrophy of the arm muscles and limitation of motion, especially in abduction, and weakness. X-ray examination showed a slight erosion of the head of the humerus. He reacted to tuberculin. An exploratory operation showed the focus to be encapsulated, and the case being considered a very favorable one for tuberculin treatment, no incision into the focus was made and the wound was closed.

We have given twenty injections of tuberculin, covering a period of five and a half months. During this time the patient's weight has remained the same. There is still slight atrophy of the pectoral and scapular muscles. The strength of the arm, however, is good and is equal to that of the other arm. There is no pain whatever in the shoulder and there are no tender points. There is practically no restriction of motion, except very slight, on abduction. He plays actively. The X-ray also shows improvement. The arm has not been kept fixed. A portion of his opsonic chart is added.

CASE V.—*Tuberculosis of hip.*

This patient, a young woman aged twenty-nine, had hip-joint disease (right hip) since thirteen years of age. She was well treated and got on fairly well, though with deformity, until 1900, when there was an exacerbation of the trouble and an exploratory operation was performed, but the joint was not opened. Within a few months the pain disappeared. One year later she suffered from tuberculosis of the right wrist, involving mainly the tendon sheaths. This was operated upon and the patient made a good recovery, with quite good functional result. The wrist has given no trouble since. Six months before coming under our observation she began to lose weight, have general malaise and to suffer from pain in the right hip and knee. For two months before we saw her the leg had been held in forced extension, and the pain had become much less. She has now been under treatment with tuberculin for three months, during which time she has had eight injections. During the past month she had had no pain whatsoever, and X-ray examination shows no evidence of any advance in the process, though the deformity due to the old trouble is so marked as to make any decision as to the extent of the present trouble difficult.





CASE VI.—*Tuberculous arthritis, ankle.*

The patient had had aching pain and swelling of the left foot and ankle for one month. On examination the ankle was found to be swollen and hot. There was marked tenderness on passive motion, and motion was considerably limited. The X-rays showed quite typical changes as commonly seen in tuberculous joints and the tuberculin reaction was positive, giving a local reaction. During a period of one and a half months he received 5 injections of the small doses of tuberculin, and during this period the swelling pain, and tenderness much improved. The patient, however, was transferred to Bayview Asylum and it was impossible to continue the treatment and observation longer.

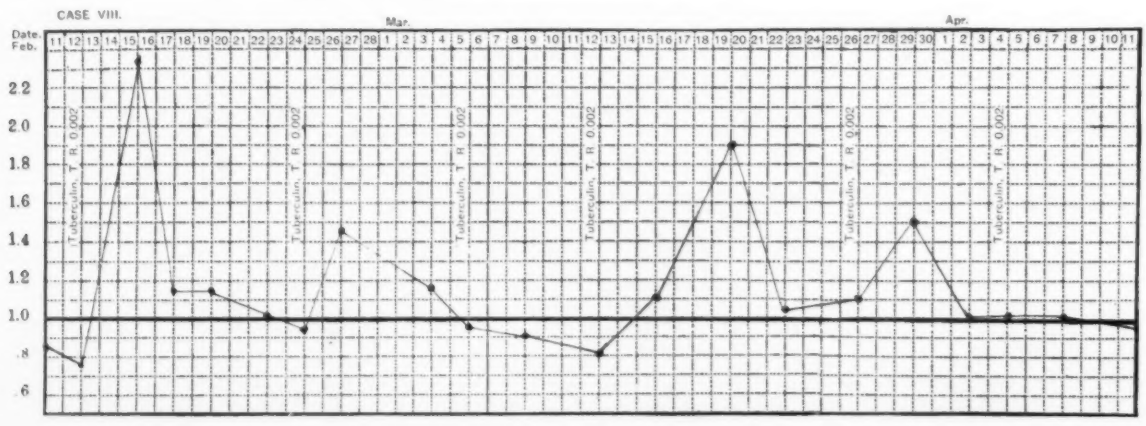
CASE VII.—The patient was an old man, aged 80 years, who for three weeks before admission had complained of swelling, pain and tenderness of the left wrist and hand. The onset was very sudden and the early stages were acute. The entire arm below the elbow had become swollen. When first seen, there was very marked tenderness, the wrist was held stiffly and there was marked local œdema. The acuteness of the process suggested some form of acute arthritis, but the X-ray examination showed conditions such as are usually seen in tuberculous joints, and he was given the tuberculin test, which was positive, with a definite local reaction. The treatment with tuberculin was started, giving as in the other cases, small doses of the tuberculin TR. infrequently repeated. Improvement seemed to begin at once. The swelling in the wrist diminished, the tenderness became less and motion in the joint slowly returned. He was kept in the hospital for a month and a half, but the joint was not fixed. At the end of this time he left the hospital, but returned for treatment. This was continued for about three months, and then was discontinued as the patient failed to return for observation and treatment. It is now four and a half months since treatment was commenced. There is still slight limitation of motion in the joint and X-ray examination shows slight erosion of the metacarpal bones, but the condition is markedly improved over what it was when the treatment was commenced. We have advised continuing the treatment and, as he is again having a little pain in the joint, he has consented and the injections have been resumed.

CASE VIII.—The patient is a boy aged 12, suffering from lupus vulgaris of about 12 years' standing. At present he has a large patch about 10 x 4 cm. under the chin and a small area about 1½ cm. in diameter on the forehead. This boy had been treated for 5 months and has received 17 injections. The lesion looks markedly cleaner and healthier and the scab formation and suppuration have much diminished. During the past month there has also been some tendency to healing about the edges of the area on the chin, but the patient states that it always becomes better in the spring. The small area on the forehead has not been influenced by the treatment.

CASE IX.—This patient was a man aged 39, suffering from tuberculous cystitis of four years' duration. He had had local treatment over a considerable period of time. He was treated for two months and received 8 injections. There was, however, no improvement in the patient's local or general condition. In fact, the local condition became worse. The patient refused to continue the treatment longer. He has since died from a tuberculous meningitis.

The study of these nine cases is sufficient, we think, to justify further continuation of the method of treatment. In none of the cases has the result been miraculous, but in certain of the cases, especially in Cases IV and VII, there seemed to be a very definite relationship between the treatment and the improvement in the patient's condition. That it is important that, in addition to the tuberculin treatment, hygienic measures should be carried out as well, is shown by a comparison of the two cases of tuberculous adenitis. The cases are too few for any general conclusions to be drawn. Other dosage and other interspacing of doses might have been more efficacious, and possibly other forms of tuberculin might give still better results. This can only be decided by the clinical results, and we feel that it cannot be decided by the opsonic curves.

We have employed the opsonic method for diagnosis in a considerable number of cases without obtaining satisfactory results. The diagnostic method described by Wright of heating the serum for the detection of immune opsonins has also not proved of value in five cases in which we have tried it. Lately, with very careful technique and with what seemed to be a very positive result, we obtained an index of 2.4 in a case that was considered to be tuberculous peritonitis, with possibly Addison's disease. Autopsy later showed no tuberculosis present, except an old calcified nodule in one lung.



STUDIES ON OPSONINS.

By WILLIAM LORENZO MOSS, M. D.

(From the Biological Division of the Clinical Laboratory.)

According to Wright, the immediate object of the vaccine treatment of disease is to so increase the opsonic content of the blood that the bacteria producing the disease may be more readily phagocyted and their noxious influence thereby more speedily ended.

The "index" of this power on the part of the serum for preparing bacteria for phagocytosis, and Wright's method of determining it, have been made familiar to all by numerous publications.

Wright has been contented to raise this index, by his inoculations, to a very slight degree, his early experiments apparently showing that a summation of positive phases (to use his terminology) such as occurs in antitoxic and bactericidal immunity does not occur in the case of opsonic immunity.

The dosage of vaccine which he has employed is purely arbitrary. Why, for instance, should 250 million streptococci be given for the initial dose rather than 2500 million? No experiments, so far as we know, have been made to determine the maximum dose, if we except certain clinical observations of Wright's, few in number, in which too large doses at too frequent intervals caused apparent summation of negative phases.

However this may be, and we feel that possibly a different interpretation may be given to his results, it has seemed important that if the treatment by vaccine is to have any scientific or practical value we must determine (a) if opsonin plays any part in immunity, (b) if we can influence its production in the body, and, if so, by what method we can obtain the most favorable results. In order that these questions may be studied a technique must be devised sufficiently accurate for the purpose and the limitations of which must be definitely known. In the following experiments we assumed that the technique of Wright was sufficiently accurate and employed it with certain additions, to be described later, in studying the above problems.

Furthermore, if the opsonins represent definite immune substances in the ordinary sense, it would seem remarkable that by the introduction of the opsinogen the increase of the antibody in the blood is so very slight. If, for instance, we could increase the opsonin in the blood 100 or 1000, or even 1,000,000 times, as is the case with agglutinin or bactericidal antibodies, this method of treatment might have much more importance.

The following experiments were undertaken to determine to what extent the opsonic content of the blood could be increased. The animals used were rabbits, the organism employed Staphylococcus aureus and control uninoculated rabbits were kept.

The method employed consisted in repeated progressive inoculations: (1) intravenously with dead staphylococci; (2) subcutaneously with living staphylococci; (3) subcutaneously with dead staphylococci.

The results were studied by Wright's method of estimating the opsonin, but it was thought that there might be an increase in the opsonin which would not be detected by this method alone; a sort of supersaturation which might only become apparent when the serum was greatly diluted. It was thought, for instance, that the serum of an inoculated animal in dilution of 1 to 100 might affect a given number of bacteria as much as an equal volume of undiluted serum of an uninoculated animal.

To determine if this might be the case the index was taken with dilutions of 1 in 5, 1 in 10, 1 in 50, and 1 in 100, as well as with the undiluted serum. The technique employed was the usual one, as closely following the method of Wright as possible.

The bacterial emulsion was prepared from a 24-hour agar growth of Staphylococcus aureus, and great care was employed to obtain homogeneous emulsions.

The various dilutions of the serum having been prepared, the preparations were made in the following way: Equal volumes of washed leucocytes, bacterial emulsion and serum (or diluted serum) were mixed in capillary pipettes and incubated at 37° C. for 15 minutes.

Films were then made from these pipettes, stained with Hasting's stain, and the average number of bacteria in each leucocyte was estimated by careful counts of 50 leucocytes on each slide.

The opsonic index was calculated in each case by dividing the number of bacteria contained in 50 leucocytes in a preparation in which rabbit's serum was used by the number of bacteria contained in a like number of leucocytes in a preparation in which a corresponding dilution of normal human serum was employed.

Record was kept of the percentage of polymorphonuclear leucocytes phagocyting in each dilution, as well as of the number of bacteria which they contained, and a curve of phagocytic index was constructed in addition to the curve of opsonic index. This phagocytic index was obtained by dividing the percentage of cells phagocyting in a preparation in which rabbit's serum was employed by the percentage phagocyting in a preparation in which a corresponding dilution of the standard human serum was used.

The records of the observations made during this series of experiments occupy 45 chart sheets and cover over 350 determinations of opsonic index, exclusive of other data collected, so that it is not feasible to include all the records with the publication of this article, but a few illustrative charts will be published and the points given which were brought out by a careful analysis of them all.

Rabbit A was a full-grown, healthy looking female rabbit, under observation from Nov. 7, 1906, to March 5, 1907, during which time it received 10 intravenous inoculations of dead staphylococci. The initial dose consisted of 125 million (approximated by Wright's method), and the dose gradually increased to 4000 millions. Observations of the opsonic index were made of the serum and each of the dilutions of the serum before mentioned, 18 times. At no time did this rabbit appear to suffer any ill effects from the inoculations.

Rabbit B was an average-sized, male rabbit, kept as a control, and at no time received any inoculations. It was under observation from November 6, 1906, to March 13, 1907. The index was taken in all the various dilutions 23 times.

Rabbit C, male, was under observation from December 5, 1906, to February 3, 1907, and received five subcutaneous inoculations of living staphylococci, the index being taken six times. Beyond slight local reaction at the sites of inoculation, this rabbit remained apparently well until it was found dead in its cage one morning. The cause of death could not be determined. Autopsy was negative as far as macroscopic examination was concerned, and cultures were negative.

Rabbit D, male, under observation from December 5, 1906, to March 13, 1907, received eight subcutaneous inoculations of dead staphylococci, beginning with 250 million and increasing to 6000 million. This rabbit showed no ill effects which could with any certainty be ascribed to the inoculations, but looked puny toward the end of the experiments, perhaps a result of the confinement.

Rabbit E, male, under observation from February 5 to March 13, 1907, received two subcutaneous inoculations of living staphylococci, and had the index taken in all the dilutions 13 times.

Chart No. 1 will serve to illustrate how the records were kept. The first two curves under date of November 7, 1906, show the percentage of cells phagocyting in the various dilutions, the solid black representing those in rabbit A's serum, the hollow dots and broken line, those in the preparation in which the standard human serum was used.

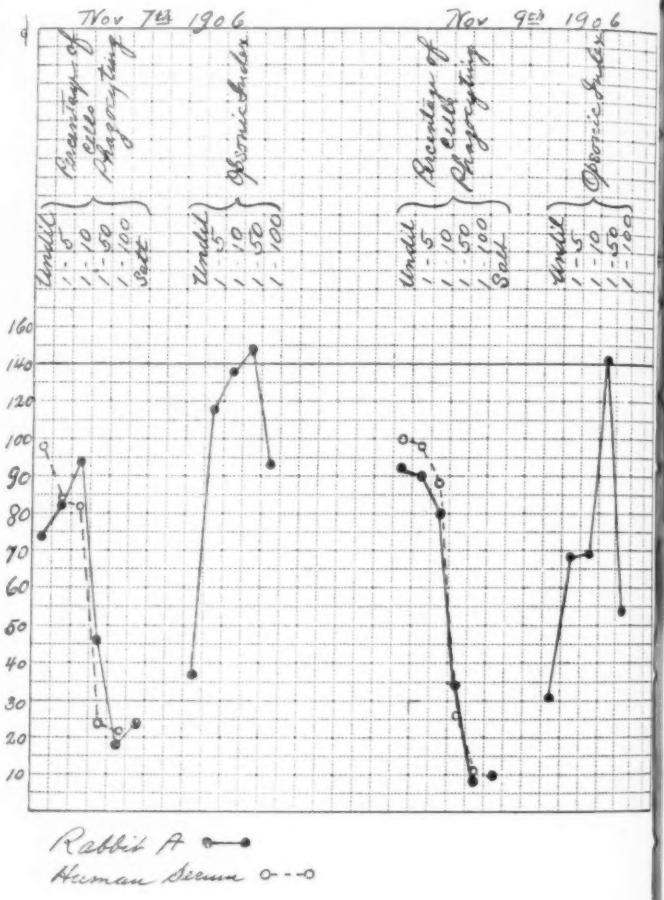
The next curve represents the opsonic index for rabbit A in the various dilutions. Similar observations are recorded under date of November 9.

From the charts, of which No. 1 is an illustration, curves of the opsonic index in all the various dilutions except 1 to 100 were constructed; portions of these curves for rabbits A and B are shown in Chart No. 2.

As previously stated, charts were made showing the curves of phagocytic index as obtained by dividing the percentage of cells phagocyting in the rabbit's serum by the percentage phagocyting in the standard serum. Chart No. 3 illustrates these curves.

From a consideration of all the curves showing percentage of cells phagocyting it is evident that in a general way the largest percentage occurred in the undiluted serum and progressively diminished as the dilution was carried on, as would be expected. There were exceptions to the rule, however, in which the percentage of cells phagocyting was greater

Chart No. 1.



in dilutions of 1 to 5, or even in 1 to 10 than in the undiluted serum. The percentage always fell between 1 to 10 and 1 to 50, though sometimes it happened that the percentage of cells phagocyting and the number of bacteria taken up was greater in the 1 to 100 dilution than in the 1 to 50.

A control preparation was regularly made in which no serum was used, but a corresponding volume of 0.85 per cent NaCl solution, and a record of the percentage of cells phagocyting in it, and the number of bacteria ingested was kept.

In this control preparation the percentage of cells phagocyting and number of bacteria ingested were generally lower than in the 1 to 100 dilution of serum, but the two results ran pretty close together and not infrequently the preparation con-

CHART No. 2.—Curve of Opsonic Index.

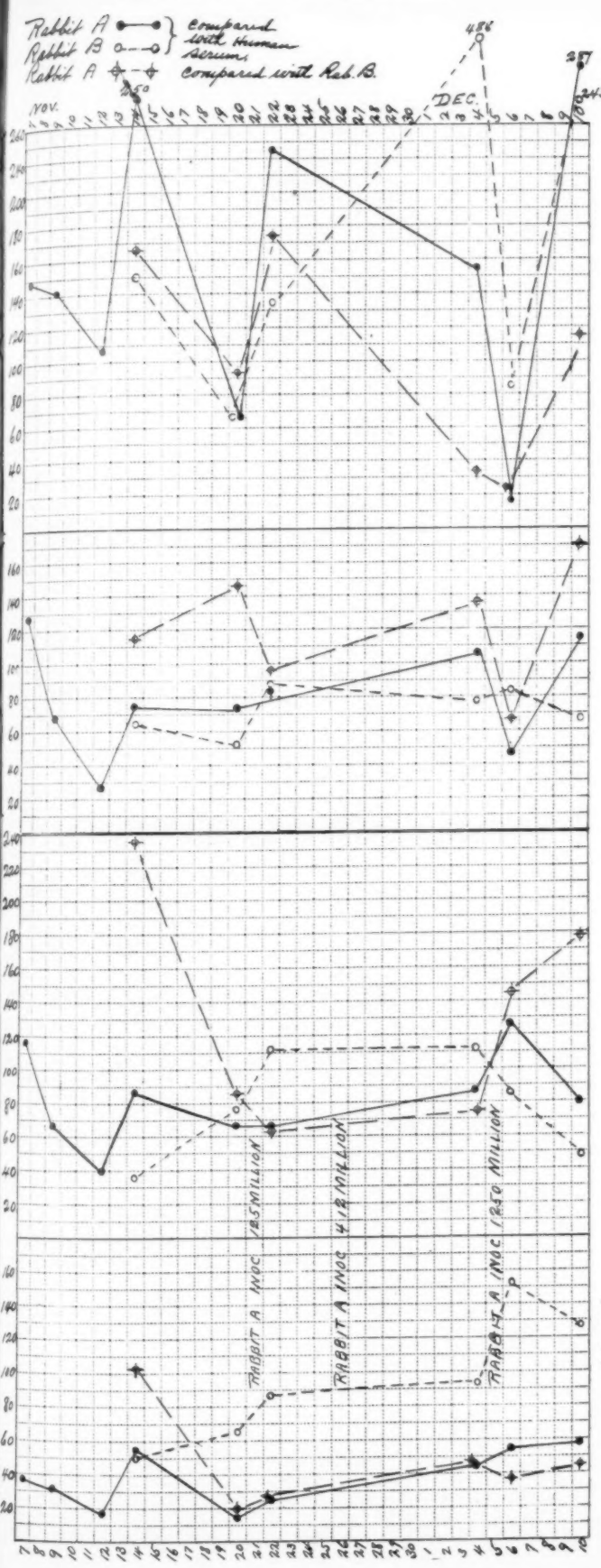
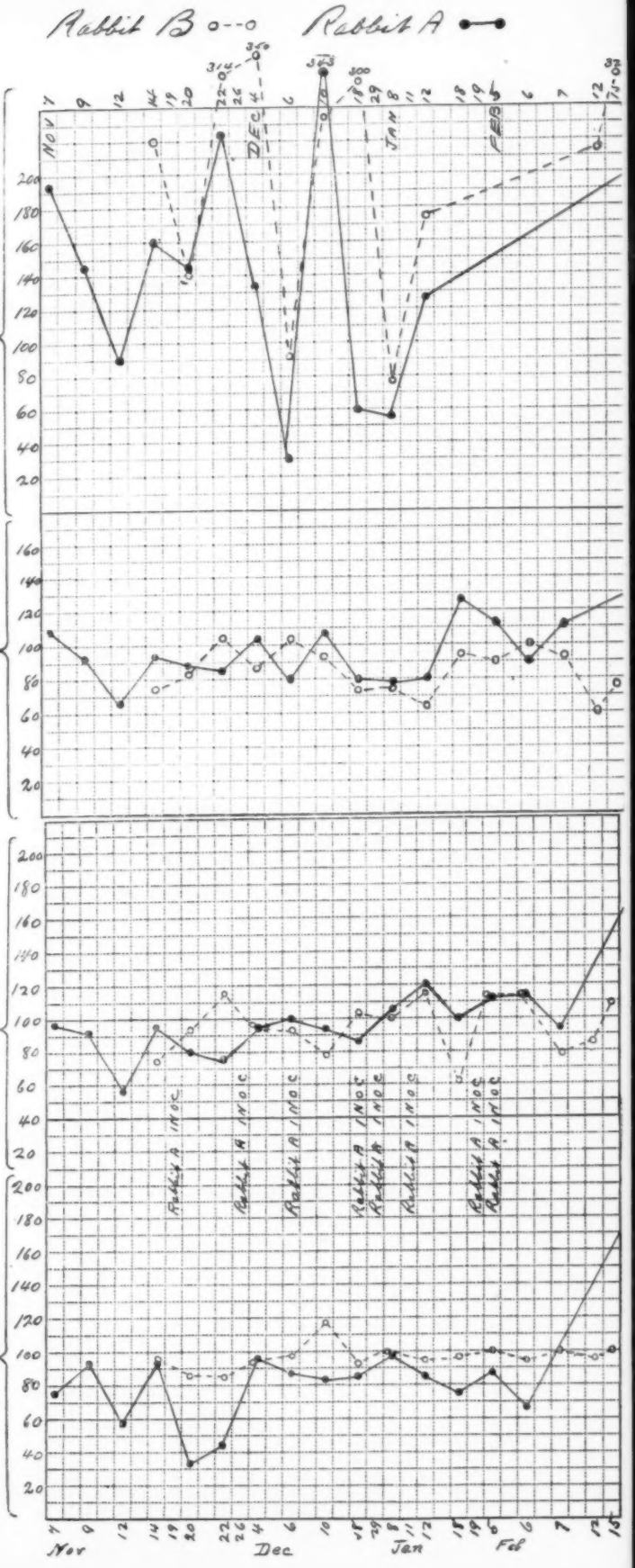


CHART No. 3.—Curves of Phagocytic Index.



taining 1 to 100 dilution of serum, or even 1 to 50 dilution, contained fewer ingested bacteria and fewer active leucocytes than did the control with NaCl solution.

Some of the above statements are illustrated in Chart No. 1, November 7, in which the percentage of cells phagocyting in rabbit A's serum increased from 74 per cent in the undiluted serum to 94 per cent in dilution of 1 to 10, then fell to 18 per cent in dilution of 1 to 100, while 24 per cent phagocyted in NaCl solution. The percentage phagocyting in the standard serum on this date follows the general rule of a progressive decline with dilution.

It may be remarked here that the absolute number of cells phagocyting in any given case seemed to depend largely on the strength of the bacterial emulsion used, and, in the lower dilutions at least, practically all of the cells could be made to phagocyte by using a sufficiently thick emulsion of bacteria, and this observation did not seem at all dependent on overlying of the leucocytes by bacteria, and is, we think, significant. This observation seems to militate against the use of the coefficient of extinction as a means of estimating opsonin.

Only in a general way did the number of bacteria phagocyted run parallel to the number of leucocytes phagocyting. Both diminished with dilution, but no constant mathematical relationship could be made out. As a rule the number of bacteria ingested diminished more rapidly than did the percentage of leucocytes.

In using bacterial emulsions of different strengths, the same percentage of cells may phagocyte, while the number of bacteria taken up may be widely different.

A study of the curves of opsonic index, of which Chart No. 2 is an example, brings out some interesting points. In the first place, four observations were made on rabbit A, November 7, 9, 12, and 14, before any inoculation was given, and it will be seen that if we accept Wright's method of determining the index as accurate, that the index of this rabbit was by no means constant, even before inoculation, the variation in the undiluted serum ranging between 0.18 and 0.56. The same variation in the index of all the other rabbits was noted where a number of observations were made before inoculation.

When we come to compare the curves of opsonic index in the various dilutions, we find, contrary to expectation, that the index as obtained in one dilution is not the same as that obtained in all the other dilutions.

It is difficult to explain this variation. If we take two sera containing different amounts of opsonin, and dilute each to the same degree, whether it be one-half or 100 fold, it seems that the opsonic content would be proportionately diluted, and that the *ratio* which one bears to the other should be the same, no matter in what dilution tested.

In the case of rabbit A the index almost regularly and with striking constancy rises with progressive dilutions, the lowest index usually occurring in the undiluted serum and the highest in dilution of 1 to 50, or 1 to 100. Chart No. 1 illustrates this fact. Reference to the curve of opsonic index on November 7 shows the lowest index 0.37 in the undiluted serum, the highest index 1.49 in dilution of 1 to 50. The curve of opsonic index for November 9 has the same characteristics, the lowest index occurring in the undiluted serum and the highest in dilution of 1 to 50. In other words the serum of this rabbit stood dilution much better than the human serum with which it was compared. This was true, though in a less striking way, of the serum of all the other rabbits. It would require too much space to include all these curves here, but they have been summarized in the following table, No. 1, which shows in what dilutions the maximum and minimum indices occurred.

TABLE No. 1.

Showing in what dilutions maximal and minimal opsonic indices occurred.

Rabbit A.	Min.	Max.
Nov. 7	Undil.	1-50
" 9	"	1-50
" 12	"	1-100
" 14	"	1-50
" 20	"	1-100
" 22	"	1-50
Dec. 4	"	1-50
" 6	1-50	1-5
" 10	Undil.	1-100
" 18	"	1-100
Jan. 8	1-50	1-5
" 12	Undil.	1-5
" 18	"	1-10*
Feb. 5	"	1-10*
" 6	"	1-10*
" 7	1-5	1-10*
" 20	Undil.	1-100
Mch. 4	"	1-100
Rabbit B.		
Nov. 14	1-5	1-50
" 20	1-10	1-100
" 22	Undil.	1-100
Dec. 4	1-10	1 50
" 6	1-10	Undil.
" 10	1-5	1-100
" 18	1-10	1-50
Jan. 8	1-50	1-5
" 12	1-10	1-5
" 18	Undil.	1-5*
Feb. 5	"	1-10*
" 6	"	1-10*
" 7	1-5	1-10*
" 12	1-10	1-50
" 15	Undil. and 1-100	1-50
" 18	1-50	1-10
" 20	1-10	1-100
" 23	1-10	1-50
" 26	1-5	1-100
Mch. 4	1-5	1-50
" 6	1-50	Undil.
" 13	1-100	"

Rabbit C.	Min.	Max.
Dec. 5	1-50	1-100
" 8	1-5	1-50
" 14	1-5	1-100 and undil.
Jan. 10	1-10	1-50
" 14	Undil.	1-50
" 18	"	1-10*
Rabbit D.		
Dec. 5	1-5	1-100
" 8	1-5	1-10
" 14	1-5	Undil.
Jan. 10	1-10	1-50
" 14	Undil.	1-50
" 18	"	1-5*
Feb. 5	"	1-10*
" 6	"	1-5*
" 7	"	1-5*
" 12	"	1-50
" 15	"	1-50
" 18	1-50	1-10
" 20	Undil.	1-100
" 23	1-10	1-50
" 26	Undil.	1-10
Mch. 4	1-5	1-100
" 6	1-50	1-10
" 13	1-10	1-100 and undil.
Rabbit E.		
Feb. 5	Undil.	1-10*
" 6	1-10	1-5*
" 7	1-5	1-10*
" 12	Undil.	1-50
" 15	1-5	1-50
" 18	1-50	Undil.
" 20	Undil.	1-100
" 23	"	1-50
" 26	"	1-10*
Mch. 4	1-10	1-50
" 6	1-50	1-100

* Dilutions higher than 1-10 not made.

As stated in the beginning of this paper, the main object of the experiments was to see to what degree the opsonic index could be raised. An examination of all the curves show that no very high indices were obtained except in dilutions of 1 to 50 and 1 to 100. The results obtained in these dilutions are not considered very trustworthy for reasons to be given

presently. The curves for each rabbit have all been summarized except the 1 to 100 dilution in Table No. 2.

TABLE No. 2.

Showing maximal and minimal opsonic indices for each rabbit during entire period of observation.

Rabbit A.	Min.	Max.	*Rabbit C.*	Min.	Max.
Undil.	.14	1.49	Undil.	.48	1.31
1-5	.40	3.55	1-5	.76	.94
1-10	.28	3.43	1-10	.64	2.39
1-50	.18	20.14 next 3.20	1-50	.50	3.22
Rabbit B.			*Rabbit D.*		
Undil.	.35	2.34	Undil.	.35	2.81
1-5	.37	2.90	1-5	.32	5.70
1-10	.31	2.75	1-10	.39	2.65
1-50	.02	4.88	1-50	.48	9.39 next 3.45

Rabbit E.	Min.	Max.
Undil.	.48	1.63
1-5	.36	2.80
1-10	.40	1.57
1-50	.40	2.90

Excepting in the dilutions of 1 to 50 and 1 to 100 the highest index obtained for any inoculated rabbit was 5.70 for rabbit D in dilution of 1 to 5. This rabbit received subcutaneous inoculations of dead staphylococci. The next highest index was 3.55 for rabbit A in dilution of 1 to 5. This rabbit was inoculated intravenously with dead staphylococci.

The control uninoculated rabbit B reached an index of 2.90 in dilution of 1 to 5, 2.75 in dilution of 1 to 10, and 2.34 in undiluted serum.

Not only did we fail to produce very high indices, but such high indices as were produced were not maintained for any length of time, and it is difficult to show any relation which they bore to the inoculations.

Furthermore, the index of the control uninoculated rabbit, with the two exceptions mentioned above, showed as great variations as that of the inoculated rabbits. See Table No. 2 showing maximum and minimum indices.

In certain places a suggestive parallelism was noted between the curves of the inoculated rabbits and the control. Instances of this are shown in Chart No. 2 in the curve of opsonic index in 1 to 50 dilution. The index of both the inoculated rabbit and the control went down to a low point on November 20 and December 6, and reached a high point on December 10.

This parallelism suggests very strongly a variation in the opsonic content of the standard serum to which the others were referred rather than a coincident increase or decrease in opsonin in the inoculated and uninoculated rabbits.

Thinking that there might be some difference between the opsonin contained in human blood and in rabbit's blood which would render indices based on a comparison of the two fallacious, the index for rabbit A was calculated, using the serum of rabbit B (uninoculated) as the standard. The curve obtained by this method differed considerably from that which resulted from a comparison with human serum, but the maximum and minimum indices fell within about the same limits, and it could not be seen that inoculation had any more definite influence on this curve than on the previous one. This curve is shown on Chart No. 2 and may be compared with that obtained with human serum as a standard.

Curves were constructed for the other rabbits based on a comparison of their sera with that of the uninoculated rabbit, but the results were not different from those in the case of rabbit A.

The results in the dilutions of 1 to 50 and 1 to 100 were considered untrustworthy, as stated above, because the index in these dilutions represent the ratio between such small numbers of bacteria; for instance, the high index obtained for rabbit A found on November 14 in dilution of 1 to 50 was obtained by dividing 25 by 10; twenty-five being the number of bacteria taken up by 50 cells in the preparation in which rabbit's serum was used, and 10 being the number taken up by 50 cells in the preparation in which human serum was used. The high index obtained for rabbit D on January 10 was the quotient of 37 divided by 15. Here it will be seen that a slight difference in either one of these numbers would make a considerable difference in the index.

As stated, curves of phagocytic index were constructed, but these seemed less reliable than those of opsonic index as a means of estimating the amount of opsonin in the blood, and space will not be taken to include all of them here.

The results of the foregoing experiments seem to cast considerable doubt on the accuracy of the methods generally employed in the estimation of the opsonic index. Wright's method seems inadequate, as do also the methods of using diluted serum, percentage of cells phagocyting, and phagocytic index as described above.

One conclusion seems justifiable, however, from these experiments, notwithstanding the inaccuracies of our methods of investigation; that is, that no very high grade of opsonic immunity can be produced by means of ordinary inoculation procedures, with staphylococci, at least, in a degree comparable to that obtained in antitoxic and bactericidal immunity.

At the conclusion of the above experiments a new series was undertaken with reference to the investigation of certain points. The culture of Staphylococcus aureus used in the previous experiments was an attenuated laboratory culture. It was decided in the new experiments to use a virulent culture and in order to obtain this, the organism previously used was passed in succession through about 10 mice. It was also decided to try a wider range in the dosage, and to control the standard serum used by making simultaneous observations under exactly the same conditions on two other normal sera. This method of control was thought to have an advantage over that of using pooled serum in that it would give information regarding variations in the different normal sera used, and, at the same time, all the advantages of pooled sera could be obtained by using the average of the three normal sera.

Four fresh rabbits were secured for these experiments, and each day for four successive days observations were made on the indices of each before any inoculation was given. Three of these rabbits, G, H, and I, had their indices taken to the same culture of Staphylococcus aureus that was used in the

previous experiments, while the fourth rabbit, J, was tested with the culture which had been passed through the mice.

The indices were first taken with reference to only one of the three normal sera, and later corrected to the average of the three. That is to say, the index was in the first place obtained by dividing the number of staphylococci contained in 50 cells in a preparation in which rabbit's serum was used by the number of staphylococci in 50 cells in a preparation in which one of the human sera was used; and later a curve was constructed in which the indices were obtained by dividing the number of bacteria in 50 cells in the rabbit's serum by the average number of bacteria contained in three counts of 50 cells each in the three human sera. A comparison of these two curves brings out no new points; they differ somewhat, but there are just as great variations in the curve based on

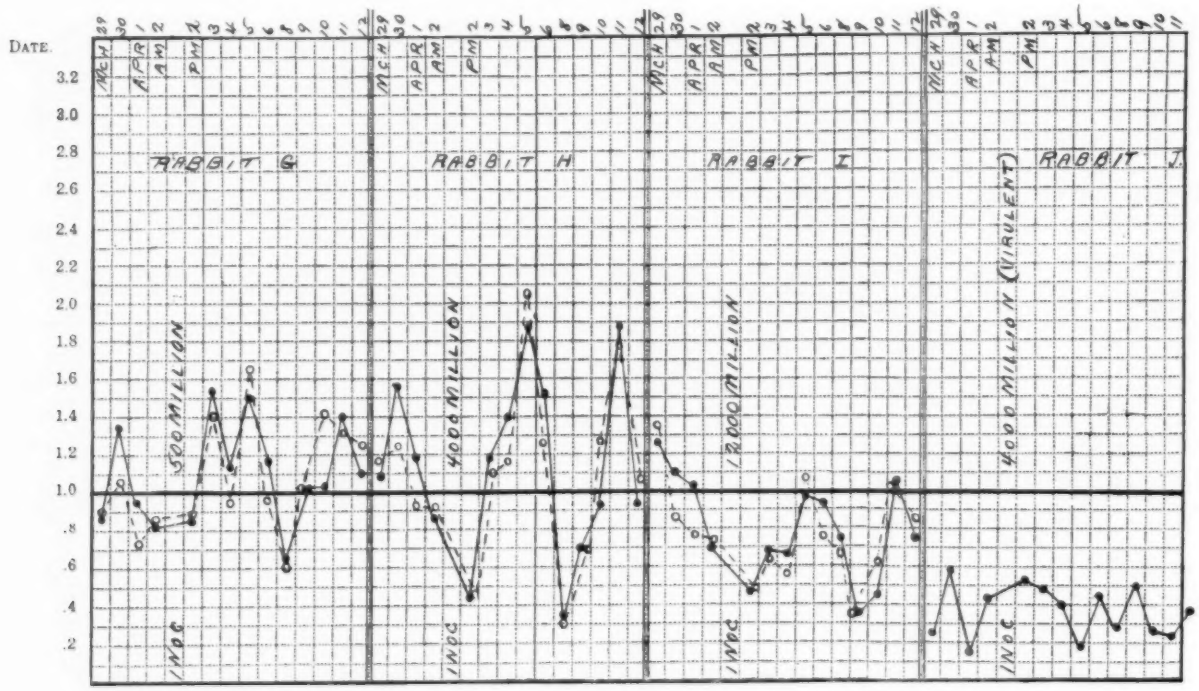
cutaneously, rabbit G receiving 500 million staphylococci from a vaccine prepared from the attenuated culture, rabbit H 4000 million staphylococci of the same vaccine, and rabbit J 12,000 million of the same. Rabbit I was given 4000 million of a vaccine prepared from the staphylococcus which had been passed repeatedly through mice.

In this series, as in the previous experiments, the indices varied in an apparently lawless way, and it seems unsafe to attempt to draw deductions from them.

Chart No. 4 shows the curves obtained for these four rabbits. The solid dots represent the indices obtained when only one of the three normal human sera was used as the basis of comparison, and the hollow dots the curve calculated on a basis of the average of the three normal human sera.

Parallel tests, as far as possible under exactly identical con-

Chart No. 4.



Solid dots show rabbits' indices referred to one of the normal sera. Hollow dots show rabbits' indices referred to the average of the three normal human sera.

the average of the three normal sera as in that based on only one, so that the first curve alone will be discussed.

In the first place during the period before inoculation the variations in the index for the different rabbits were as follows: Rabbit G, 0.81 to 1.34; rabbit H, 0.87 to 1.57; rabbit I, 0.70 to 1.26; rabbit J, 0.14 to 0.59. During the same period the variation of two of the normal human sera as compared to the third, the third being that used in determining the indices for the rabbits, was 0.70 to 1.66 in the case of one and 0.84 to 1.95 in the case of the other. After these preliminary observations, all of the rabbits were inoculated sub-

ditions, were made on the three normal human sera. The curves obtained by calculating the indices for two of these as tested against the third are shown in Chart No. 5.

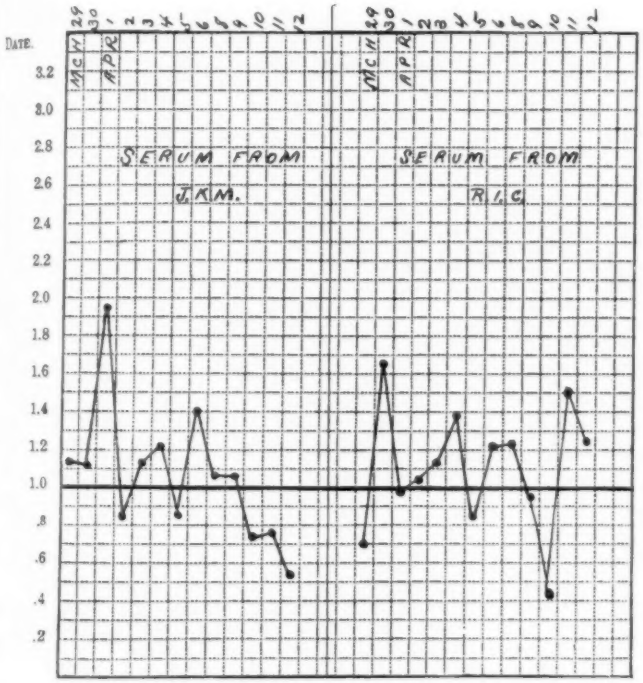
These curves are most irregular. In 13 observations one varies between limits of 0.52 and 1.95, the other between 0.42 and 1.66.

We do not expect the index to exhibit much wider variations under pathological conditions than these three normal sera show. Hence if the above results are trustworthy it would not seem possible to make use of the opsonic index as a means of diagnosis or as an indication for the treatment by

vaccine. No attempt will be made here to discuss the clinical value of the vaccine treatment. The existence of opsonin and the fact that it plays a part in phagocytosis seems well established, but we think it remains to be proven that it plays any part in immunity, or that it can be used as a means of diagnosis or as an indication for treatment.

If the normal serum varies in its opsonic content through as wide limits as pathological serum does, no amount of smoothing out these variations by pooling or striking averages will enable us to say that one opsonic index indicates infection or immunity while another does not.

CHART No. 5.



In view of the discordant results obtained in the above experiments it seemed wise to determine if they might be due to personal error on the part of the observer. Accordingly a series of comparative tests were arranged to be carried out by three observers simultaneously, and as far as possible under exactly the same conditions, all these workers having had considerable experience with the technique.

For this purpose each of the three observers prepared three specimens of his own serum. These were given to a fourth man, who numbered them and kept a record of their source, so that the observer might not know with which serum he was dealing in a given test, until all the tests were made and the results obtained. An emulsion of fresh leucocytes and one of a 24-hour agar culture of Staphylococcus aureus was prepared, great care being taken to secure homogeneous emulsions, and, in the case of the bacterial, free from clumps. As soon as these were ready each emulsion was apportioned equally between each of the three observers, who immediately set to work putting up one preparation from each of the nine specimens of serum. Thus there were 27 preparations made in all on sera from three normal individuals.

The preparations were each incubated 15 minutes at 37° C. Spreads were made, stained with Hasting's stain, and 50 leucocytes and their contained bacteria were counted on each slide.

The results are shown in the accompanying Table No. 3.

TABLE No. 3.

April 13, 1907.				Average.	Difference between highest and lowest counts.	Greatest absolute and percentage variation from average counts.
	C's serum.					
	1	2	3			
A's counts	238	224	197	219	41	19 or 8.6%
B's "	164	185	264	206	100	58 or 28%
C's "	241	252	177	223	75	46 or 20%
	B's serum.					
	4	5	6			
A's counts	190	203	255	218	59	37 or 17%
B's "	340	310	234	295	106	61 or 21%
C's "	189	319	211	273	130	84 or 30%
	A's serum.					
	7	8	9			
A's counts	216	217	236	223	20	13 or 5.8%
B's "	213	211	291	238	80	53 or 22.4%
C's "	289	215	178	227	111	62 or 27.3%

Attention must be drawn to several points in this experiment. First we are dealing with three different sera which may contain different amounts of opsonin. In the second place, we have three different observer's results on the same serum, and here may be introduced personal differences, which, while they may be constant for each man and so not affect a comparison of his own results at one time with his results at another, would introduce an error in comparing his results directly with those of another observer. Thirdly, we have three observations by one observer on one serum.

Perhaps it is best to begin our examination of the results of this test by considering the third point first. The greatest variation between the three counts by any one man on one serum was C's counts on B's serum. His highest count was 319, lowest count was 189, a difference of 130. The greatest absolute and percentage variation from the average of his three counts on B's serum being 84 or 30 per cent.

The smallest variation between the three counts of any one man on one serum was A's count on A's serum, in which the highest and lowest counts were 236 and 216, respectively, a difference of 20; the greatest absolute and percentage variation from the average of his three counts being 13, or 5.8 per cent. It is evident from a comparison of these figures and from subsequent work done that a considerable error results from counting too few cells, 50 cells not being a sufficient number to yield a true average.

For the reason stated above (variation due to personal differences in making the counts) it does not seem wise to attempt any comparison between the results of the different ob-

server's observations on the same serum, although in this experiment they come out fairly close together, except in the case of B's serum, in which A finds the low average of 218 while B finds the high average of 296. The difference between individuals in these observations on the same serum in subsequent tests were much greater.

We now will consider the first point, namely, the differences between the three sera used, and here again it is perhaps not wise to consider the averages of the three different observers for the three different sera, but compare the averages of one observer on the three sera. Thus A's averages for the three sera are very close, 218 to 223, while B's varied from 206 to 295.

This experiment was repeated on the following day, using a much thicker bacterial emulsion. The results are shown in Table No. 4. Here a striking difference is noted between the results gotten by the different observers on the same serum.

TABLE No. 4.

April 14, 1907.				Average.	Difference between highest and lowest counts.	Greatest absolute and percentage variation between highest or lowest and average counts.
	C's serum.					
	5	6	8			
A's counts	921	931	943	932	22	11 or 1.1%
B's "	515	621	557	564	106	57 or 10%
C's "	647	548	603	599	99	48 or 8%
	B's serum.					
	2	3	7			
A's counts	941	903	1023	956	120	67 or 7%
B's "	665	821	975	720	454	255 or 35%
C's "	725	750	609	695	141	84 or 12%
	A's serum.					
	1	4	9			
A's counts	903	946	935	928	43	25 or 2.7%
B's "	651	790	697	713	139	77 or 10%
C's "	813	728	463	668	350	205 or 30%

The thicker emulsion makes counting very much more laborious, but probably yields more accurate results, provided the emulsion used is not so thick as to introduce error by causing bacteria to overlie leucocytes without being really in them, or causing the number in the leucocytes to be so great as to render accurate counting impossible.

After each of the previous tests were made the slides were given to a fourth man who kept record of them, but renumbered them, and after mixing them up, issued them again to the different observers who made recounts.

On the recounts of the slides of the first experiment, differences varying from 1 to 69 were made on the individual slide, with an average difference for the 27 slides of 29. The recounts on the slides of the second test showed considerably greater absolute variations, but perhaps the percentage variation was not much greater, as the bacterial emulsion used in the second test was much thicker, and we are therefore dealing with much larger numbers.

Further tests were performed in which from six to nine preparations each were made from one serum, the bacteria contained in 50 cells being enumerated on each preparation. The results will not be detailed here, but they strengthened the conviction that the present technique is inadequate. The problem now was to find out in what points the error lies. Not much more than a start has been made along that line, but it seems worth while to state what has been done and indicate the lines along which further investigation might be made, hoping that those who are working in opsonins will at least test their technique, and, if it is found deficient, be saved from wasting further time on a method which yields only inaccurate results.

That phagocytosis faithfully follows definite laws seems probable. That opsonin exists and plays a part in phagocytosis we are equally agreed. That many factors influence phagocytosis we are certain. Whether we can control enough of these factors to study the process in an exact and scientific way may be open to question.

An effort was made to control as many of the factors influencing phagocytosis as possible in the following tests.

Fresh leucocytes were washed and great care was taken to get a homogeneous emulsion. The same care was resorted to in the preparation of the bacterial emulsion. A very fine capillary pipette was used to reduce as much as possible the effect of settling of the bacteria and leucocytes during incubation; the serum, bacterial, and leucocytic emulsions, in equal parts, were mixed very thoroughly before incubating and again after incubating before making the spreads for counting. Light staining was used in order that all the bacteria within a cell, even those overlying the nucleus, could be counted, and then a number of counts of 50 cells each was made on *one* slide. It seemed that if these counts all made on the same slide varied greatly that the technique certainly could not be applied to the investigation of different preparations in which the number of variable factors could not possibly be as small as in this one preparation.

The results of 12 counts of 50 cells each made on one slide will illustrate what we found. They were: 331, 422, 391, 388, 287, 367, 412, 489, 418, 495, 371, 503 given in the order in which they were made, beginning away from the end of the smear and counting down to the end.

It seems quite evident from a comparison of these 12 counts that 50 cells do not suffice to give us a true idea of the averages. In these 12 counts we have a variation of from 287 to 503.

An attempt was made to get an idea of how many cells one would have to count to find a true average, and for this purpose these 12 counts were combined in groups of 100, 150, 200, and 300. The averages for these different groups are given on a basis of the content of 50 cells, as this furnishes a convenient means of comparison.

The averages for the six groups of 100 cells each are as follows: 377, 389, 327, 450, 457, 437. For the four groups of 150 cells each the counts are 381, 347, 439, and 456. For groups of 200 each the counts are 383, 388, 447. For groups of 300 each the counts are 364 and 448. The

average for the 600 cells is 406, and we are probably justified in assuming that this represents more closely the true average than any of the smaller groups. On this assumption let us calculate the greatest absolute and percentage variation in each of the smaller groups. In the groups of 50 each this greatest variation from the average for the 12 counts is $406 - 287 = 119$, or 26 per cent. In groups of 100 each it is 79, or 19 per cent; in groups of 150 each, it is 59, or 14+ per cent; in groups of 200 each it is 41, or 10 per cent; in groups of 300 each it is 42, or 10 per cent. As far as the result of this one test goes it shows that we can reduce the error by counting larger numbers of cells, but that we still have an error of, in this case, at least 10 per cent, in counting as many as 300 cells.

The impression prevailed among the men in the laboratory that the count was dependent in part upon the place on the slide from which the cells were counted, although there was difference of opinion as to where the counts would be greatest. The counts just described seemed to throw some light on this question, as the counts were begun some distance back from the end of the smear, and were continued moving toward the end. An examination of the counts shows in general that the highest counts occurred toward the end of the slide, particularly is this evident if the larger groups are considered.

There seems a probable explanation for this. The smears were made in the way familiar to all, of putting a drop of the mixture from the pipette near one end of a slide and with the end of another slide, held at an angle to the first, drawing the fluid along the length of the slide. There is a distinct sorting out process of the cells by this method, the smaller ones being the first to slip from under the end of the second slide and remaining behind on the first slide. Thus, in examining such a preparation one usually comes first to the lymphocytes, and though the sorting out process is not so effectual among the large mononuclears and polymorphonuclears, it is evident to some extent here also.

It seems possible that those polymorphonuclears containing a large number of bacteria may be so increased in bulk or consistence as to be spread out on the slide at a different place from those containing fewer bacteria. In order to discover if this might be true slides were divided into different zones and counts of 150 cells made in each of these zones.

The zones were 1 cm. wide and the results of the counts on two slides so determined, in connection with the 12 counts on one slide referred to above, indicate that counts made near the end of smears are slightly larger than those made near the beginning.

The results for 150 cells in each zone were as follows:

	a	b	c	d
Slide No. I.....	454	484	484	
Slide No. II.....	288	306	362	404

To summarize briefly the conclusions which these experiments seem to justify:

1. No high degree of opsonic immunity such as is possible in antitoxic and bactericidal immunity, can be produced in rabbits by ordinary inoculation procedures with Staphylococcus aureus.

2. None of the present methods of estimating the opsonic content of the blood seem sufficiently accurate to be of practical value.

It seems important that further effort should be expended in finding a reliable technique for clinical purposes rather than in continuing to pile up statistics which are so inaccurate as to be misleading.

THE OPSONINS IN TYPHOID IMMUNITY.

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Through the discovery of opsonins new protective bodies have been found, which apparently are of great importance in immunization against infectious diseases, and it is therefore interesting to determine to what degree these bodies are active in typhoid fever in which the agglutinins and bactericidal substances form in large amounts. He who believes in a purely humoral typhoid immunity, and holds the opinion that in the body fluids of typhoid patients bactericidal substances develop in sufficient quantity to kill the bacteria without the agency of phagocytes, might expect that opsonins, which prepare bacteria for phagocytosis, would be of less or no importance in typhoid immunity. The rôle, however, which agglutinins and bactericidal substances play in typhoid immunity is doubtful. All attempts to make a prognosis in typhoid from the development of these bodies must be considered unreliable. The numerous observations of Jörgensen[1] show that the agglutinating power usually declines after the third week. A high agglutinative power does not at all protect against relapses or recurrences; and the same may be said of the bactericidal substances, if determined by the Neisser[2] method. Stern and Korte[3] report that in a patient whose serum revealed the highest bactericidal titre (1:4000000) ever observed by them, a relapse developed eight days later. Other authors also have observed that a blood of high bactericidal power does not protect against recurrence. The bactericidal substances, as demonstrated by the Neisser method, diminish like the agglutinins

[1] Centralbl. f. Bakteriol. u. Parasitenk., Jena, 1905, XXXVIII, 1 Abt., 475.

[2] Berl. klin. Wchnchr., 1904, XLI, 213.

[3] Ibid.

towards the end of the disease, and especially in convalescence.[4 5] But this refers only to the bactericidal action *in vitro*. Other results were obtained if the bactericidal power was measured by the Pfeiffer method. Töpfer and Jaffé[6] comparing the bactericidal power *in vivo* and *in vitro*, in a large number of typhoid patients and immunized animals, obtained very surprising results. Working with the Neisser method, they found, like Stern and Korte, a high bactericidal power during the active stage of typhoid, and a weaker one during convalescence. But with the Pfeiffer method they found that the serum of convalescents was more effective than that of acutely ill typhoid patients. Immunized animals never showed *in vitro* such high bactericidal power as typhoid patients; tested by the Pfeiffer experiment it was the reverse. The authors do not explain this; they only express their belief that it is not due to differences in the active bactericidal substances.

Little study has been made hitherto of the typhoid opsonins. Wright and Douglas found the typhoid bacillus "eminently unsusceptible" to the opsonic action of the blood fluids. On the other hand, Leishman, Harrison, Smallman, and Tulloch[7] decided from their work that, if typhoid opsonins existed, their presence could not be demonstrated by the method they adopted. They found no changes in the opsonic power during the course of the immunizing process. Hektoen[8] mentions that in typhoid convalescents the opsonins are greatly increased, and that the opsonic index often reaches as high as four.

If it is true that the typhoid opsonins only develop to a marked degree during convalescence, then the results of Töpfer and Jaffé may possibly be explained by the fact that in the Pfeiffer experiment they injected immune opsonins and thereby caused a much more active phagocytosis, which determined the survival of the animal. In favor of this explanation are the experiments of Besredki,[9] who injected typhoid immune serum with typhoid bacilli into the peritoneal cavity and observed an increased phagocytosis which, however, he thought was due to the action of stimulins. Töpfer and Jaffé found also a stronger extracellular bacteriolysis in the exudate of highly immunized animals. But this may be explained by the fact that, if there is a stronger phagocytosis, a smaller number of bacteria outside the cells are exposed to the influence of the bactericidal substances; and a smaller number of bacteria are more readily killed by these substances than a larger one.

It was my intention to contribute to the solution of these questions by the simultaneous observation of agglutinins and the bactericidal and opsonic powers of the serum in several cases of typhoid fever. But because of lack of time, I have confined myself to experiments with immunized rabbits. I also counted the leucocytes to look for relations between the humoral and leucocytic reactions, and to determine whether there occur in the immunized rabbit changes in the blood picture similar to those observed in cases of human typhoid fever.

Agglutination was tested by the macroscopic method with the usual precautions. The result was determined after one hour's incubation. For the bactericidal power Wright's method was first tried, but the results were very inconstant. That this is due to the method is obvious from the fact that Wright[10] himself gets very contradictory results. He, for instance, examined the same healthy man twice and found the first time a bactericidal power of 3120 (the serum killed 3120 bacteria in 24 hours), while the second time he obtained a value of 6,000,000. Besides he does not consider in his method the possibility of diversion of complement. In order to complement the whole amount of amboceptors in a typhoid serum, it is often necessary to add more than a hundred times as much normal serum, and consequently, using Wright's method, I had to work with a serum at least 100 times diluted, and in order to discover smaller values, I had to resort to special experiments without adding complement. Leishman and his co-workers also do not consider the possibility of diversion of complement. This is a great error, since numerous experiments (Neisser and Wechsburg,[11] Töpfer and Jaffé,[12] Korte and Steinberg[13]) have shown that in high immune sera there is rarely present a sufficient amount of complement to satisfy the amboceptors attached to the bacteria, and to produce thereby the bactericidal effect. Highly immune sera without addition of complement very often have no bactericidal power at all.

It is hardly probable that what has almost always been observed by German and French investigators, should never have been found by Leishman and his co-workers. And if we submit the curves of these authors to a closer analysis we find that in all probability the Neisser-Wechsburg phenomenon was present. On the seventh to the ninth day after the first inoculation there was a distinct increase of bactericidal substances. The second inoculation was performed on the eleventh day. The effect was a more rapid rise of the bactericidal curve. But three days later there begins a sudden, steady decrease to a subnormal value; then the curve rises again to higher values. The authors are unable to account for this, and yet a diversion of complement would offer a full explanation. The fall of the curve four days after the second inoculation is probably not to be explained by a decrease of bactericidal substances. On the contrary it is probably due to a marked production of amboceptors, so that more and more complement is bound to that portion of the amboceptors not attached to bacteria.

I have considered these results of Leishman fully in order to demonstrate the weakness of Wright's method in its present form. The simplicity also of the latter's method is only apparent. I found that I could work very much faster with

[4] Berl. klin. Wchnschr., 1904, XLI, 213.

[5] Korte and Steinberg. Deutsches Arch. f. klin. Med., Leipz., 1905, LXXXII, 321.

[6] Ztschr. f. Hyg. u. Infectionskrankh., Leipz., 1906, LII, 393.

[7] J. Hyg., Cambridge, 1905, V, 380.

[8] J. Am. M. Ass., Chicago, 1906, XLVI, 1407.

[9] Ann. de l'Inst. Pasteur, 1901, XV, 209.

[10] Proc. Roy. Soc. Lond., 1903, LXXI, 54.

[11] München med. Wchnschr., 1901, XLVIII, 697.

[12] Ztschr. f. Hyg. u. Infectionsk., Leipz., 1906, LII, 393.

[13] Berl. klin. Wochenschr., 1904.

the Neisser method, as modified by Stern and Korte for the sero-diagnosis of typhoid fever. More important still, this method leads to more exact results. In this method to a constant mixture of bacteria and fresh serum (complement) there is added inactivated typhoid serum in varying quantities As a measure of the amboceptors present, I took that dilution of the serum, which after three hours' exposure at 38° C. produces a definite decrease of the colonies as compared with the effects produced by the fresh normal serum. To meet the objection that the results obtained by this method might be due to agglutination alone, it may be said that these two reactions often do not run parallel, and that the serum frequently exhibits bactericidal powers in much higher dilutions than it does agglutinating. The simplest proof against this objection, in my opinion, can be obtained by the following control test, in which heated serum without complement is added to the bacteria. The agglutinins are not destroyed by the process of inactivation, and their effect in the original experiment cannot be stronger than in this control test. In my experiments I have used frequently such a control test with a serum dilution of 1:10, and I have observed only a very slight diminution of colonies as compared with the agglutination power. The effect of the agglutinins on the number of colonies was less than the influence of the normal serum, in a dilution of 1:15, which was used as complement. This may be explained by the fact that after the agglutinins have been absorbed by the bacteria, the new generations of bacteria may develop without being agglutinated.

Practically the technique as described by Stern and Korte was used, but I found two slight modifications advantageous. First, I did not make the dilutions of the bacteria with broth, but with normal salt solution. The reason for this is that the bacteria when transferred to new broth, develop rather quickly even at room temperature, and therefore irregularities in the number of inoculated bacteria may occur. In order to obtain an equal quantity of broth in each tube the dilutions of the normal serum were made with broth. Second, I inoculated the tubes with a smaller amount of bacteria, in order to have all the bacteria more quickly killed, instead of causing mere reduction in their number. I used 0.1 cc. of a 24-hour broth culture, dilution 1 to 8000. In the whole series of experiments, but one broth, all made at the same time, was employed. The mixture was made in long test tubes, and before plating, the agar was poured into them. The tubes were then carefully shaken and the contents poured into the plating dishes.

Greater difficulties were found in the determination of the *opsonic power*. The typhoid bacillus, after being taken up by the leucocyte, is very quickly digested. Even after a very short time of incubation (for instance, five minutes), bacteria in all stages of digestion are seen, from the well stained, clearly outlined bacillus to a hardly visible, pale shadow (Hastings' modification of Jenner stain). Therefore, in determining the phagocytic index, the question arises, how many bacteria may be already digested and consequently invisible? Because of this fact, it must be expected that a comparative count of phagocyted typhoid bacilli can not be attempted without considerable error. One might hope to reduce these errors by a shorter incubation time. However, that would not be advisable in the determination of the opsonic index, since due to slight differences in the diameter of the capillary pipettes, unequal heating would follow if the incubation time were too short. In order to avoid these disadvantages, it was very natural to apply an analogous method to that used for the determination of agglutinins, namely, to determine *in what degree of dilution the serum ceases to cause phagocytosis*. By this method very definite results were obtained. Of course, as in all absolute measurements, some precautions must be carefully observed. First, the choice of the test-bacillus is of great importance. As shown by Löhlein,[14] the individual qualities of a given strain are of marked influence on the resulting phagocytosis, and on the action of opsonins. Löhlein, for instance, found among three different strains of B. coli one that was opsonized very readily; the other two were but little influenced by normal serum. One of them was very susceptible to phagocytosis, without any opsonic action by the serum; the other one was not taken up at all, even under the influence of serum. According to Löhlein the virulent bacteria generally have the greatest resistance to phagocytosis.

In order to get good results with the above-mentioned dilution method, it is best to use a strain which is not susceptible to phagocytosis without preparation by opsonins. The typhoid strain used by me for the opsonin test was of this kind. It was grown directly from the blood of a typhoid patient. In normal salt solution, as well as in the highest dilutions of the serum, there was no phagocytosis at all, or only an average of one-half to one and a half bacilli per leucocyte. However, as it would be necessary with such a small degree of phagocytosis to count a very large number of leucocytes in order to obtain accurate results, I took as measure of the opsonic power, the dilution in which the number of phagocyted bacteria surpassed 0.5 per cell. When a bacillus is used which is susceptible to spontaneous phagocytosis, a control test with salt solution must always be made. The measure of the opsonic power is then that dilution in which phagocytosis considerably exceeds spontaneous phagocytosis. Usually I made the test in dilutions of 1:2, 1:4, 1:8, and so on. Several times I have examined in this way the serum of three normal rabbits. In these cases the phagocytosis nearly always dropped below 0.5 per cell when a serum dilution of 1:16 was used. In a dilution of 1:8 the phagocytosis just surpassed 0.5 per cell. As in making this test there occurs a further three-fold dilution by adding equal quantities of bacterial emulsion and leucocytes, the expression of opsonic power is 24.

The influence of the number of bacteria used in the opsonin test is important. Apparently in a given culture the bacteria differ in the readiness with which they are opsonized, some being easily opsonized, others only with very great difficulty. The normal opsonins are only able to opsonize the

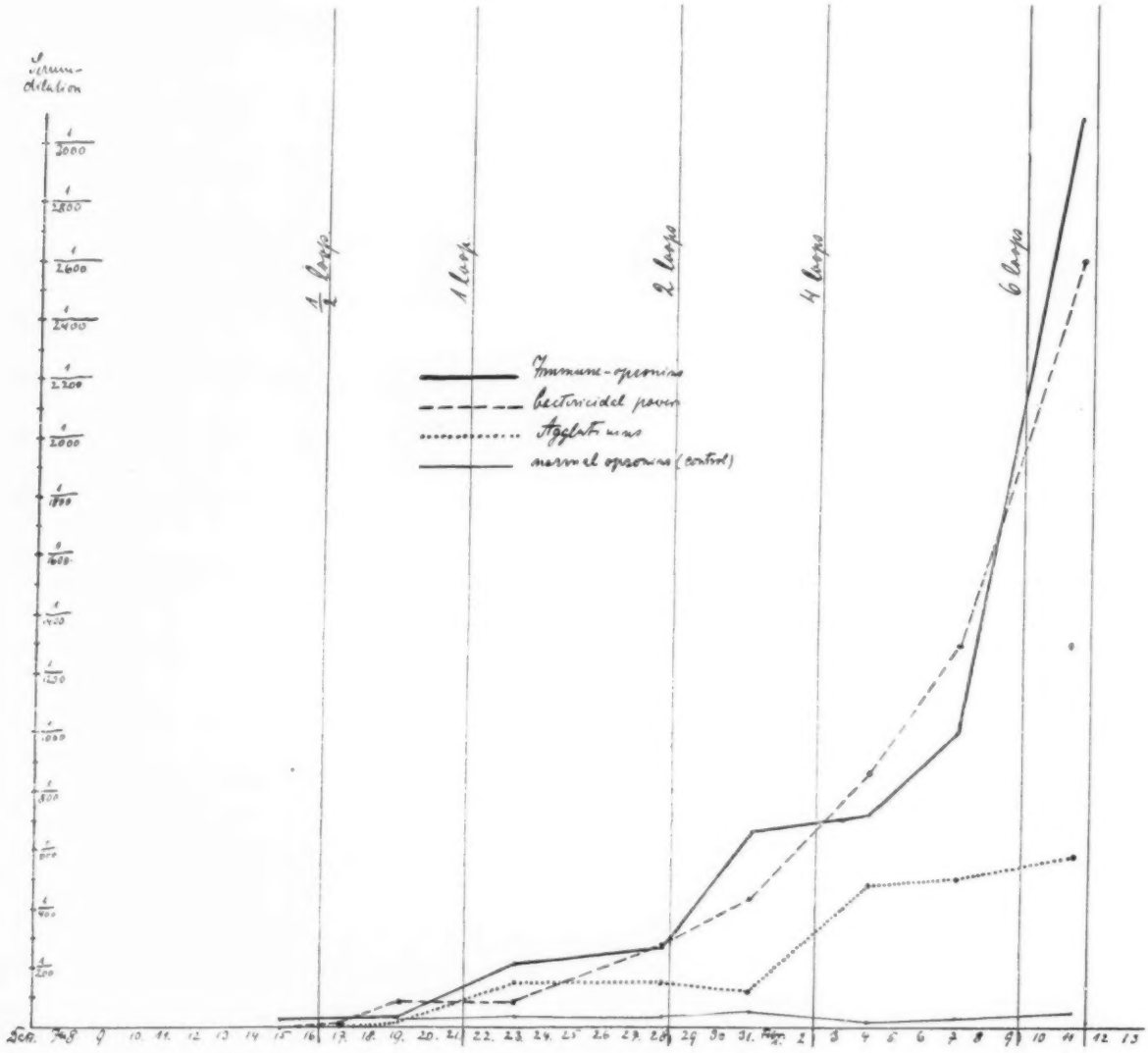
[14] Ann. de l'Inst. Pasteur, 1906.

susceptible organisms. As the opsonic power becomes higher, more resistant bacteria are opsonized.

To obtain accurate results with the dilution method it is necessary to employ rather thick bacterial emulsions of constant density. I found it convenient to use a suspension of three loops of a 24-hour agar culture in 1 cc. salt solution. It is advisable to control the degree of concentration by a standard emulsion. The incubation time in my experiments was 10 minutes; however, 5 minutes would possibly be better. The blood was taken every morning between 11 and 12 o'clock, before the rabbit had taken any food. At the same time the blood count was made.

The number of the leucocytes may have a certain influence on the result. If a given number of opsonized bacteria are mixed with a small number of leucocytes every leucocyte will take up more bacteria than if the same number of opsonized bacteria are distributed among a larger number of leucocytes. However, this influence does not seem to be very considerable, as is shown by the following experiment: To two tubes, equal parts of bacterial emulsion and leucocytes were added, but in one of them the leucocytic emulsion had been diluted 10 times. Each leucocyte from this tube contained 21.6 bacteria; from the first one, 11.6 bacteria.

The curve below shows the development of the opsonins, the agglutinins and the bactericidal substances under the influence of increasing doses of living typhoid bacilli, subcutaneously injected.



This curve was constructed in the following manner: As a measure of the opsonic power, not only the highest dilution in which the phagocytosis just surpassed 0.5 was taken but a more accurate determination was attempted in the following way: If the degree of the first positive phagocytosis was

just about 0.5, the corresponding dilution itself was taken as a measure of the opsonic power. If, however, the first positive phagocytosis was considerably higher than 0.5, the value was considered nearer to the next higher dilution. How that is expressed in the curve is best shown by an instance.

On January 31 and on February 4 the highest dilution showing positive phagocytosis was 1:384. But the phagocytic index in this dilution was very different in the two cases. On January 31 there was 0.66 bacterium per leucocyte, on February 4, 4.2. On February 4, therefore, the opsonic power was considerably higher than on January 31. The phagocytic index in the next higher dilution was but a little less than 0.5, namely, 0.42. For measuring the opsonic power, it was now calculated what percentage of 0.5 the phagocytic index was in the first dilution in which it was less than 0.5. The corresponding percentage of the difference between the last dilution with positive phagocytosis and the first dilution with negative phagocytosis was added to the last dilution with positive phagocytosis, and the number so obtained was taken as an expression of the phagocytic power. In the above-mentioned instance the determination was done in this way:

The phagocytic index in dilution $1/384 = 4.2$
The phagocytic index in dilution $1/768 = 0.42$
$0.42 = 84\%$ of 0.5
84% of $384 = 323$
Therefore the opsonic power $= (384 + 323) = 707$.

I realize that these figures are not accurate, but represent in a general way the increase of the amount of opsonins in the serum.

In constructing the curve of bactericidal power, it was not possible to rectify the values in a similar manner. However, I tried to express in my curve whether the first clear reduction of colonies was relatively slight or marked. In the case of a slight reduction, the corresponding dilution itself was taken as the expression of the bactericidal power. In the case of a very strong reduction the middle point between the corresponding dilution and the next higher dilution was taken.

Since in the arrangement of my experiments, 0.5 cc. complement and 0.1 cc. bacterial emulsion were always added to 1.0 cc. of the diluted serum, the number representing the degree of serum dilution must still be multiplied by 1.6 in order to get the real value of the bactericidal power.

The curve (page 248) shows that at the beginning of immunization the three protective substances increased at about the same rate. Later there was a rapid increase in the opsonic and bactericidal power, but only a *very* slight increase in the development of agglutinating substances. It is very striking that the dilutions in which the first increase of opsonic and bactericidal effect is found are nearly the same. The average ratio between the dilutions in which opsonic and bactericidal action ceased was $\frac{1.04}{1}$.

However, in comparing the absolute values, it must be remembered that the opsonins were determined after an incubation of ten minutes, the amboceptors after three hours, and the agglutinins after one hour.

By these experiments it has been shown that by *immunizing a rabbit against typhoid, opsonins are formed in a considerably high degree.* When we take into consideration the fact that the opsonic effect was obtained in ten minutes, we may suppose that the opsonins may be the most active substances of the typhoid immune serum. Therefore, it is possible *that the preparation of bacteria for phagocytosis, and the phagocytosis itself, may be at least as important for immunization against typhoid as the increase of bactericidal action of the blood serum,* which by many is now considered the most important factor.

The fine line of the curve shows the opsonic power of the serum of a normal rabbit, which was always examined as a control at the same time and in the same way as the immune serum. There occur only slight oscillations. Even these oscillations, however, are apparently only physiological variations of the opsonic power of the normal blood. The fact that corresponding oscillations are to be seen in the curve of the immune opsonins shows that these variations depend chiefly, if not entirely, upon irregularities of the technique. Therefore, the curve of the immune opsonins should probably have a more regular course and coincide still more closely with the amboceptor curve.

In spite of the high amount of opsonin present, the *opsonic index* examined by the original method of Wright (with a thin bacterial emulsion) on repeated examinations always showed a low value: On January 4, 1.65; on January 7, 1.04; on January 11, 0.82.

This seems to show *that the opsonic index as determined by the Wright method cannot be a measure of the opsonic power of a typhoid immune serum, at least not in the higher grades of immunity.* The index seems to rise with the first development of immunity; with the further development of immunity, it drops. I can find no other explanation for this than the fact *that the amboceptor laden bacteria are much more quickly digested by the leucocytes,* and thereby become invisible. Leishman and his co-workers, who also found a low phagocytic index in the sera of typhoid patients, tried to explain it by an intracellular bacteriolytic action of the serum. This explanation cannot be accepted in my experiments, because very numerous bacteria, staining normally, were always seen outside of the cells, and because *destruction of the complement by heat had no influence on the phagocytic index.*

During the first days after the injection of bacteria, *a more or less marked leucocytosis occurred.* It was due chiefly to an increase of the polymorphonuclear neutrophilic cells. I did not find an increase of the large mononuclear leucocytes, as is so often found in typhoid fever.[15] The eosino-

[15] Thayer: Johns Hopkins Hosp. Reps., Balto., 1900, VIII, 487.

philic cells likewise did not show any considerable variation in number.

CHART I.

Injections of living typhoid bacilli.	Date.	Agglutination.	Bactericidal power.	Opsonic power.	Normal opsonic power of control.	Index of Wright.	Leucocytes, total.	Polymorph. neutrophilies (absolute number).	Mononuclears.
	Jan. 8		<16 *						
	" 9		<16 *						
	" 12						8000	2000	5000
	" 13		<16 *				8500	2800	4900
	" 14						8600	2800	5300
	" 15	5	<16 *	<30					
½ loop	" 16	5					8400		
	" 17	5	>16				10900	6900	3900
	" 18						14700		
1 loop	" 19	>10	96	34	24		8800		
	" 23	160	96	204	43		25400	15900	9300
2 loops	" 28	160	288	284	40		9300	4400	4300
4 loops	" 31	120	432	669	52		12700		
	Feb. 4	480	864	708	10	1.65	11600		
6 loops	" 7	500	1296	909	29	1.04	9700	3900	5300
	" 11	575	2592	3072	58	0.82			

* Probably = 0, as 1:16 was the lowest dilution (for the curve taken as = 0)
The double black lines correspond to the injections of bacteria.

Because of the high amount of opsonin in the immune serum, it was easy to study the peculiar qualities of these opsonins. First, I was able to show that the typhoid immune opsonins are thermostabile, resisting heating to 55-58° C. for half an hour. The average numbers of bacteria contained in a leucocyte are shown in Table I.

TABLE I.

	With unheated serum	With heated serum.
Dilution 1/48	∞	∞ *
Dilution 1/192	2.87	2.32
Dilution 1/768	0.63	0.53
Dilution 1/3072.....	0.21	0.06

Salt solution, 0.15.

* Leucocytes crowded with bacteria in all stages of bacteriolysis.

According to this table, the opsonic action disappeared in the same dilutions of the heated and unheated serum, and was of an equal power in corresponding dilutions. On the contrary, the opsonin of the normal serum was thermolabile under the same conditions.

TABLE II.

	Unheated serum.	Heated serum.
Dilution 1/1	..	1.12
Dilution 1/6	14.3	1.18
Dilution 1/12	4.7	0.2
Dilution 1/24	0.65	0.04
Dilution 1/48	0.2	0.05

Salt solution, 0.0; (70 cells).

However, the table shows that the opsonin of the normal serum does not disappear entirely after heating for half an hour to 56° to 58° C. Whether this small amount of thermostabile opsonin is identical with the thermostabile immune opsonin, cannot be decided until further experiments have been made. These experiments were repeated with the same results.

In order to find out whether the typhoid immune opsonin is specific or not, the opsonic index of the immune serum for other bacteria was first determined. Next I tried to absorb the immune opsonin by saturation with other bacteria. The opsonic index for other bacteria was also determined, not only with an unheated normal serum, but also with a heated one. By determining the index with heated sera, non-specific action of the typhoid immune opsonin can apparently be shown much better, because of the thermostability of the immune opsonin. The results are given in the following table:

TABLE III.

Phagocytic Index.	Typhoid immune serum.		Normal serum.	
	Unheated.	Heated.	Unheated.	Heated.
Against Staphylococcus aureus	7.5	0.63	6.7	0.51
Against Streptococcus..	6.2	2.4	6.7	1.8
Against Bacillus tuberculosis *	1.9	0.2	1.9	0.29
Against guinea pig erythrocytes	Traces		Traces	

* These specimens were kindly made by Mr. Sellards.

The typhoid immune serum had no higher opsonic power for the other organisms tested than had normal serum, and by heating, this opsonic power was destroyed in the same degree as the opsonic power of the normal serum. Therefore, it is evident that *the typhoid immune opsonin is specific*, at least against the organisms studied. Also, human erythrocytes were not opsonified by the immune serum in a higher degree than by normal rabbit serum.

By saturation with some other micro-organism no immune opsonin could be absorbed. The phagocytic index of the immune serum against typhoid bacilli was:

	Before saturation with staphylococci.	After saturation with staphylococci.
In a dilution of 1:243.....	7.2	7.4

Also by saturation with guinea pig corpuscles the opsonic power of the immune serum for typhoid bacilli was not diminished.

Contrary to the immune opsonin, *the opsonin of the normal rabbit serum proved to be largely non-specific.*

In order to determine to what degree the normal opsonin can be absorbed, it is necessary to use very large quantities of bacteria and a concentrated serum. With this purpose, several agar cultures were emulsified in a few drops of normal salt solution. It is necessary, of course, to employ a strain easily opsonized. This suspension was mixed with an equal part of normal rabbit serum and drawn into capillary

pipettes. These were sealed and incubated for some time; in the first experiment for fifteen hours, in the second one for three and three-quarters hours. At the same time the part of the serum which was to be used for the control was also sealed in a capillary pipette and incubated. Then the staphylococci were removed. In the first experiment they were thrown down by the centrifuge, in the second they were filtered out by a very small Berkfeld filter. Then the opsonic index of the clear fluid was tested. In the first experiment there were still some staphylococci in the clear fluid. However, there was no trouble in distinguishing the typhoid bacilli and staphylococci within the leucocytes. Some doubtful organisms were taken for altered typhoid bacilli. The phagocytic index was:

TABLE IV.

	Experiment I.	Experiment II.
With normal serum.....	5.9	3.9
After absorption with Streptococci..	0.23	0.09
With salt solution.....	0.17	0.09

As shown by this table, *the opsonin of the normal serum can be absorbed completely by other bacteria. Therefore the above-mentioned thermostabile opsonin of the normal serum is apparently not identical with the typhoid immune opsonin.* Table III also shows that in the typhoid serum there is *no increased production of opsonins for other bacteria in addition to the typhoid opsonin.* This possibility must be considered, as Hektoen [16] has shown the presence of hæmopsonins in cases of typhoid fever.

That the typhoid immune serum did not contain non-specific stimulins is seen by the fact that the activity of the leucocytes toward other bacteria was in no degree favored by the typhoid serum. I mention this, because Leishman and his co-workers [17] claim to have induced a considerable production of stimulins by vaccination with typhoid bacilli. The first sentence of Leishman's description of his stimulin test shows why he was mistaken. He gives this definition of stimulins: "Substances which appear to stimulate phagocytosis, but differ from opsonins in being thermostabile." The more recent observers generally agree that the immune opsonins, which are identical with the "bacteriotropic substances" of Neufeld and Rimpau,[18] are thermostabile. It is, therefore, evident that Leishman and his co-workers really determined the amount of typhoid immune opsonin by their stimulin test.

I will not speak of all the possible reasons why Leishman failed to find an increase of opsonins following typhoid vaccination. The main cause, in my opinion, is that he used an old laboratory strain of bacteria, which apparently was little susceptible to opsonic action. However, I can only surmise this, because in Leishman's paper there is nothing said about the strain used in regard to its resistance to phagocytosis in normal salt solution.

The question arises whether the immune opsonin is formed by the transformation of the normal opsonin under the influence of the infection, or whether it is an entirely new substance. As the opsonic power of the typhoid serum for other bacteria is not reduced (*v.* Table III), and as furthermore the normal opsonins for typhoid bacilli and staphylococci are apparently identical, it must be concluded that *in the typhoid serum, in addition to the immune opsonins, the non-specific normal opsonins are present in normal quantities.* Therefore it is probable that the *immune opsonin* is not a transformation of the normal opsonin, but a *definitely new substance.*

Similar results to those quoted in the above experiments were obtained by Neufeld and Hühne. I am very sorry I did not see this paper of Neufeld and Hühne,[19] which was read in 1906 at a meeting of the "Freie Vereinigung für Biologie," until my experiments were finished and the greater part of this paper was written. I publish my paper in its original form, because I consider it important that different observers have independently obtained similar results, and because these results do not agree with those obtained by Leishman.[20]

Concerning the question whether the opsonins are different from other protective substances, my experiments do not offer much new evidence. However, there are some points of interest. In the highest dilutions in which an opsonic action could be found, no agglutination of bacteria was seen, even in the smears, and the phagocyted bacteria were distributed singly in the leucocytes. This cannot be considered conclusive evidence against the identity of agglutinins and opsonins, as the same substances which agglutinate the bacteria in a certain concentration might in a much higher dilution possibly be able to opsonify the bacteria. A more positive proof against this identity is given by the *different course of the curves of agglutinins and of opsonins.*

The parallel course of the opsonin and amboceptor curve is very striking, as is also the fact that both substances cease to be active in the same dilutions. And further, as both substances are thermostabile and specific, the suspicion arises that they may be identical. But even though this parallelism be a rule, it can not be considered a positive proof of the identity of these substances, as it is quite possible that two substances, which are apparently very similar and are produced under the same conditions, may be formed in equal quantities. Neufeld and Rimpau give some important reasons against this identity, but they acknowledge that a positive proof of non-identity has not yet been advanced, at least in the case of typhoid fever.

Finally, it must be asked whether these results can be useful for diagnosis. I have given my reasons why the index taken in the ordinary way cannot be considered a valuable method in typhoid fever. An examination with the above described dilution method requires too much time, though

[16] J. Am. M. Ass. Chicago, 1906, XLVI, 1407.
[17] L. c.
[18] Deutsche med. Wchnschr., 1904, XXX, 1458.
[19] Centralbl. f. Bakteriol., 1906, Bd. XXXVIII, Abtl., 27 Beil.
[20] Neufeld and Hühne's complete paper has just appeared in Arb. a. d. k. Gsndhtsamte., Berl., 1907, XXV, 164.

far less than the determination of the bactericidal power, which has been advised for diagnostic purposes.

I examined the opsonic power in only two cases of typhoid infection. The first one was a case of typhoid fever in about the seventh week. Here the serum in a dilution 1:120 caused a very marked phagocytosis, whereas the control serum in a dilution 1:30 caused less phagocytosis, and with the control serum diluted 60 times, there occurred no phagocytosis at all. The opsonic index was 1.02.

The second case was one of cystitis, due to a typhoid-like bacillus. The patient had had typhoid fever many years ago. The serum of this patient still caused a very marked phagocytosis in a dilution 1:288 (phagocytic index 4.8). Higher dilutions unfortunately were not made, because such a high opsonic power was not expected. The control serum caused but slight phagocytosis in a concentration of 1.36 (phagocytic index 0.66). The bacillus isolated from the urine had the cultural qualities of B. typhosus.

In my opinion it will be most advisable to study for diagnostic purposes the different effects of heating on the normal opsonins and the immune opsonins. As the normal serum contains only traces of thermostabile opsonins, it will be useful to look for them in a dilution of about 1:30. Perhaps it will be possible to differentiate the thermostabile opsonin of the normal serum from the immune opsonin, and to shorten the method by heating the serum for a shorter time and at a higher temperature.

With this method the differences between the normal and the typhoid serum must be very great. It will not be necessary to detect small differences in the number of the phagocytic bacteria, the determination of which takes too much time, and is open to so many opportunities for error.

STUDIES ON THE SPECIFICITY OF OPSONINS IN NORMAL AND IMMUNE SERUM.

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In the various papers of Wright and his collaborators to which I have had access, the question of specificity of the opsonins is nowhere discussed, though everywhere it is apparent that they consider the opsonins specific. This is very evident from the fact that they have used the opsonic index for diagnostic purposes, and as this would have no value unless the opsonins were specific, I think we may safely assume that they believe the question of specificity to be settled.

From the clinical results obtained by Wright and others by the administration of vaccines controlled by the opsonic index, it has seemed evident that the increase of opsonin present in the patient's serum is specifically related to the germ used. Moreover, according to Wright, the opsonic index to the organism causing the infection or used as a vaccine, is often abnormal, while with other organisms it varies very little from that of the control serum. On clinical grounds, therefore, the assumption of specificity of the opsonins would seem justified.

As this clinical method of arriving at a solution of the question is difficult to control, and as patients may be suffering from mixed infections, the question has been approached in a different manner by using the absorption method which Ehrlich introduced for the purpose of showing the specificity of amboceptors, etc., in immune sera.

The first study on the subject of specificity of opsonins was that of Bulloch and Western (1); they used normal serum and stated that there was a high degree of specificity of the opsonins as shown by their experiments with staphylococcus, B. pyocyaneus and B. tuberculosis.

They were able to show that after saturation of normal blood with staphylococcus, nearly all of the opsonin for that organism had been absorbed, while the opsonins for B. pyocyaneus and B. tuberculosis were largely retained.

This work, so far as I know, has not been confirmed. Simon (2) comes to quite opposite conclusions and states that there is no indication of the specificity of opsonins. He uses quite a different method from Wright in obtaining his index of opsonic power. Wright obtains his phagocytic index by averaging the number of organisms in a certain number (20 to 40) of leucocytes, while Simon neglects the average number of organisms per leucocyte and states as his result the percentage of leucocytes which phagocyte. He has made parallel tests with these methods and states that they give approximately the same results (5). It should be noted that he used B. typhosus, B. coli and staphylococcus in his absorption experiments, whereas Bullock and Western used B. tuberculosis, B. pyocyaneus and staphylococcus.

Potter, Ditman and Bradley (3) repeated Bulloch's experiments, using B. coli and staphylococcus, and they also failed to confirm the findings of Bulloch and Western. They found that saturation of normal serum with staphylococcus removed not only the opsonin for that organism, but also removed the opsonin for B. coli and vice versa.

In my experiments with normal human and normal rabbit serum, and these experiments have been repeated many times, I have uniformly found that saturation with staphylococcus removed not only its own opsonin, but also that for B. coli.

Normal rabbit serum was saturated at 37° C. for 30 minutes with staphylococcus aureus, and the mixture was then filtered

to remove the bacteria. The phagocytic index was obtained with a sample of the serum before and after saturation.

NORMAL RABBIT SERUM.

	Phagocytic Index.	
	Before saturation with Staphylococcus.	After saturation with Staphyloccus.
Staphylococcus	1.76	0.13
B. coli	0.94	0.15

This experiment was reversed by saturating with B. coli instead of with staphylococcus.

NORMAL RABBIT SERUM.

	Phagocytic Index.	
	Before saturation with B. coli. 1½ hrs. at 37° C.	After saturation with B. coli.
B. coli	13.66	...
Staphylococcus	3.38	0.30

Here it is seen that saturation with B. coli removed nearly all the opsonin for staphylococcus, and that saturation with staphylococcus removes the opsonin for B. coli.

These results were uniformly true whatever the length of time during which the saturation was carried out (the duration of saturation was varied from 30 minutes to over 13 hours) and regardless of whether the saturating process was carried out at 0° or 37° C.

As these results were quite the opposite to those obtained by Bulloch and Western, and also at variance with the theory of the specificity of the opsonins which Wright everywhere assumes, and which the clinical side of opsonic work seems to justify, I made a further series of experiments using the same organisms that were used by Bulloch and Western in one of their tests, and while my results varied slightly at different times and witih different sera, the results may be looked upon as confirmatory of those of Bulloch and Western in that saturation with staphylococcus did not remove the opsonin for B. pyocyaneus to any extent.

NORMAL HUMAN SERUM.

	Phagocytic Index.	
	Before saturation over night at 37° C. with Staphylococcus.	After saturation.
Staphylococcus	2.80	0.46
B. pyocyaneus	10.26	8.13

This of itself seems to indicate that Bulloch and Western were right in asserting the existence of a high degree of specificity of opsonins. The reversal of the experiment, however, did not give the same results. Normal rabbit serum was saturated with B. pyocyaneus at 37° C. for two hours. The mixture was then filtered. The filtrate was tested and at the same time a test was made of the unsaturated serum, and it was found that B. pyocyaneus had reduced the phagocytic index for staphylococcus from 3.7 to 0.7. It seemed possible from this that B. pyocyaneus might be an exception to the general rule, so the following experiment was done.

Rabbit serum immune to guinea-pig corpuscles was divided into two portions, one of which was heated to 56° C. for 30 minutes. A portion of the heated serum and one of the unheated serum were then saturated with guinea-pig corpuscles and the phagocytic index to staphylococcus, streptococcus and B. pyocyaneus obtained.

RABBIT SERUM IMMUNE TO GUINEA-PIG BLOOD.

	Phagocytic Index.			
	Unheated.		Heated.	
	Before saturation with guinea-pig corpuscles.	After.	Before saturation.	After.
Staphylococcus	1.06	0.19	0.00	0.00
Streptococcus	2.48	0.46	0.14	0.03
B. pyocyaneus	7.06	2.71	3.75	3.02

This I think shows that B. pyocyaneus, at least the strain which I have used, is an exceptional organism in its behavior to opsonins, in that heating the serum does not destroy its opsonic power for this organism.

A further experiment was made to find out whether B. pyocyaneus has any other peculiarities.

NORMAL HUMAN SERUM BEFORE AND AFTER SATURATION AT 37° C. WITH STAPHYLOCOCCUS AUREUS FOR ONE AND ONE-HALF HOURS.

	Phagocytic Index.				
	Dilution 1 in 2.		Dilution 1 in 4.		
	Before saturation.	After saturation.	Before saturation.	After saturation.	Control containing no serum.
B. pyocyaneus	15.83	7.82	5.76	3.60	3.64
B. typhosus	3.81	0.15	1.60	0.14	0.23
B. coli	3.43	0.10	0.30	0.00	0.00
Staphylococcus aureus	2.48	0.52	0.81	0.38	0.64
Staphylococcus albus	4.66	1.00	1.56	0.70	0.00

This experiment shows that B. pyocyaneus is phagocyted spontaneously to a much greater extent than any of the other organisms tested, but that phagocytosis takes place better when serum is added; and it shows that the stronger the serum the more marked is the phagocytosis. In addition, it shows that the opsonin of normal serum for the entire list of organisms, including B. pyocyaneus, can be reduced or removed by saturation with staphylococcus aureus.

Bulloch's experiment with B. pyocyaneus was as follows: He saturated normal serum with staphylococcus, precipitated the cocci in the centrifuge, giving a supernatant fluid "A." A portion of this was then saturated with B. pyocyaneus, again placed in the centrifuge and a supernatant fluid "B" obtained.

	Phagocytic Index.	
	Staphylococcus	B. pyocyaneus.
Normal human serum 1: 2.....	22.9	4.7
Normal human serum 1: 4.....		3.0
Normal human serum 1:2. After saturation with staphylococcus. Fluid A	0.5	4.0
Fluid A after saturation with B. pyocyaneus = Fluid B.....		0.4

The experiment was not reversed or results such as I have found might have been obtained. Such a result as in the first experiment is perfectly possible when B. pyocyaneus is used, for it undergoes spontaneous phagocytosis to a considerable extent, though the phagocytic index of normal serum for this organism is somewhat reduced by saturation with it. We can offer no explanation for the result obtained by Bulloch in the second experiment with Fluid B.

As to the specificity of the opsonins in normal serum for tuberculosis, I have not as yet been able to make any experiments.

Muir and Martin (4) have obtained results analogous to ours in that they were able to remove the opsonins for staphylococcus from normal serum by saturation with a combination of antigen and the corresponding amboceptors, as precipitins or lysins. They find that in their behavior under these circumstances the opsonins resemble complement.

Thus, it is seen that the results of Simon, Potter, Ditman and Bradley, Muir, and Martin, and my own all speak decisively against the specificity of the opsonins of normal sera, and I believe I have found a possible explanation for the opposite results of Bulloch and Western in their pyocyaneus experiment in the fact that B. pyocyaneus is readily susceptible to spontaneous phagocytosis.

During the course of these experiments it was thought possible that in the opsonic reaction complement or a non-specific complement-like substance might be necessary to activate the haptophoric groups which might be specific, and that therefore the apparent non-specificity of opsonins might be due to an insufficient amount of this complement or similar body, so that saturation with any variety of bacteria would remove all of this complement, leaving the haptophoric group for other bacteria, however, intact. A series of saturations at 0° C. were therefore carried out, but with quite negative results, in that the opsonins for both organisms were removed as in the saturations at 37° C. The bacteria were removed by filtering at 0° C. From these experiments at low temperature, it seems probable that the complement takes no part in opsonization.

Two experiments were done with immune sera—one a rabbit immunized to guinea-pig corpuscles, and the other a rabbit immunized to B. typhosus. The results of the first are given in the following table:

RABBIT SERUM IMMUNE TO GUINEA-PIG CORPUSCLES.

	Phagocytic Index to Guinea-pig Corpuscles.		
Dilutions.	Before saturation.	After saturation with guinea-pig corpuscles.	After saturation with streptococcus.
1-4	+	trace	+
1-8	+	—	+
1-16	—	—	—
1-32	—	—	—

	Phagocytic Index to Streptococcus.		
Dilutions.	Before saturation.	After saturation with guinea-pig corpuscles.	After saturation with streptococcus.
1-4	13.80	2.50	2.0
1-8	3.3	0.8	1.0
1-16	1.0	0.8	0.3
1-32	1.6	0.4	0.6

As the average number of erythrocytes ingested by the leucocytes is difficult to determine accurately, the method of dilution was used in the same manner as in agglutination tests. It will be observed that the phagocytosis of erythrocytes in the unsaturated serum disappeared when a dilution of 1 in 16 was reached; saturation of this serum with the antigen reduced the phagocytosis in a dilution of 1 in 4 to a mere trace, while on the other hand, saturation of this serum with streptococcus did not effect the immune opsonin for the antigen. This is evidence that the opsonin of this immune serum was specific. The second part of the table shows the behavior of the opsonins for a non-antigen—streptococcus. It is quite clear that there is here no question of specificity of the normal or non-immune opsonin, as practically as much opsonin for streptococcus is removed by saturation with guinea-pig corpuscles as by saturation with streptococcus itself.

The serum from a control rabbit showed entire absence of opsonin for guinea-pig erythrocytes.

A rabbit serum immune to B. typhosus contained sufficient opsonin to cause phagocytosis of typhoid bacilli, when tested in one of Wright's pipettes, in dilutions up to 1 in 1000, while phagocytosis with normal rabbit serum ceased in dilutions of 1 in 8 to 1 in 16. This serum was saturated with guinea-pig erythrocytes in the usual way, without any effect whatever on the immune opsonin for its antigen—B. typhosus.

A number of comparative observations by Dr. Meakins of the opsonic indices for several organisms following the injection of a vaccine of one of them for clinical purposes, apparently shows that the opsonin for the antigen is much more increased than that for the other organism. This was well demonstrated in sera following injection of meningo-coccus vaccine, which caused a greater rise in the opsonic curves than did any other vaccine which has been employed in the laboratory. Such sera did not show corresponding increase of opsonins for Staphylococcus aureus or Staphylococcus albus. We feel, however, that, owing to the errors inherent in the method, absolute conclusions cannot be drawn from such charts.

CONCLUSIONS.

(1) Following injections of bacterial vaccines, the increase of opsonins is probably due to the formation of immune opsonins which react specifically.

(2) In saturation experiments with normal sera we are dealing with normal or common opsonins, and these are not specific, since they can be removed by saturation with any one of a considerable number of bodies.

(3) In saturation experiments with immune sera we have both the immune and the common opsonin present; the former is quite specific, while the latter is not.

(4) B. pyocyaneus is quite susceptible to spontaneous phagocytosis.

We would further state that our conclusions are based on experiments which have been repeated a number of times, and that the results were so conclusive as to convince us that they do not merely represent differences due to the errors inherent in the Wright method.

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THE METHOD OF "FIXATION OF COMPLEMENT" IN THE DIAGNOSIS OF MENINGOCOCCUS AND GONOCOCCUS INFECTIONS.

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The phenomenon of "fixation of complement" has been well known since Bordet and Gengou (1) demonstrated that complement, added to a mixture of inactivated bacteriolytic serum and bacteria would not produce hæmolysis when sensitized erythrocytes were subsequently introduced. But it remained for Neisser and Sachs (2) to first make a practical application of this principle. They employed it in order to recognize human blood in medico-legal investigations. The method used by them may here be described briefly. A mixture of erythrocytes with the inactivated serum of an animal immunized to them, is spoken of as a hæmolytic system. When to such a system fresh complement is added, hæmolysis occurs. But if the complement should first be added to a mixture of the inactivated serum of an animal immunized to human serum, plus a trace of human serum, and then subsequently be added to the above hæmolytic system, no hæmolysis occurs. That the trace of human serum is necessary for this phenomenon to occur may be demonstrated by omitting it, and then hæmolysis will proceed as before. Neisser and Sachs explained this phenomenon by supposing that the human serum contains receptors, and that the inactivated serum immune to human serum contains amboceptors which bind the complement to the receptors, and thus prevent its action in the hæmolytic system. They also found that the smallest trace of human serum, at times even 0.000,001 cc., was sufficient to produce this phenomenon. Then Wassermann and Brück (3) applied this principle, not to detect the presence of specific serum albumins, but to detect the presence or absence of small amounts of dissolved bacterial products (antigens) or their corresponding anti-bodies in serum or inflammatory exudates. The first disease studied by them was typhoid. When an extract of typhoid bacilli (dissolved bacterial substances), typhoid patient's serum and complement were mixed and incubated for a short time, it was found that the complement would not reactivate a hæmolytic serum. Thus it was concluded that the patient's serum contained amboceptors for the dissolved bacterial substances. They also demonstrated the presence of bacterial substances in the serum. If to the serum of a typhoid patient, the inactivated serum of an animal highly immunized to typhoid bacilli, and fresh complement be added, it is found that hæmolytic serum is again not reactivated by the complement contained in the mixture. In other words, the complement has been bound. We may therefore conclude that the patient's serum contains dissolved bacterial substances (bacterial receptors) or antigens. These writers then applied this method to the study of tuberculosis and the tuberculin reaction (4). Their first step was to demonstrate the presence of anti-tuberculin in tuberculous organs. But in the blood of untreated patients no anti-tuberculin could be demonstrated. Following the administration of tuberculin, however, anti-tuberculin could be detected in the blood. It is very unfortunate from a diagnostic standpoint that tuberculin or anti-tuberculin is not present in the blood of untreated patients with localized tuberculosis. Brück (5), however, has made a very interesting observation on the blood of a patient suffering from general miliary tuberculosis. He found that in the early stages of the disease tuberculin was present; but during the course of the disease this disappeared and anti-tuberculin could be demonstrated. Before the lethal termination, however, the tuberculin was again detected. Here we have a method of diagnosis for the early stages of miliary tuberculosis, a disease which is notoriously uncertain in its early manifestations.

About the same time Wassermann, Neisser, and Brück (5) investigated the possibilities of this method for the diagnosis of syphilis. The syphilitic origin of general paresis and locomotor ataxia was likewise investigated by studying the cerebro-spinal fluid of suspected patients. The antigens were obtained from the organs of a syphilitic fœtus (see below) and the anti-bodies (amboceptors) from the inactivated serum of an immunized ape.

Besides the above writers, Wassermann and Plaut (7) Neisser, Brück and Schught (8), Bab (9), Schütze (10), Marie and Levaditi (11), Morgenroth and Stertz (19), and P. Mühlens (12), have reported similar investigations concerning syphilis. Müller and Oppenheim (13) have studied one case of gonococcus infection, and Brück (14), two cases of meningococcus and one case of streptococcus infection. Neufeld and Hüne (15) have made application of the method in typhoid and para-typhoid fever and hog cholera, and Neisser and Sachs (16) and Schütze (17) have further reported on its use in forensic medicine.

TECHNIQUE.

As previously stated, the object of the test is to detect the presence or absence in the serum investigated of (a) the antigen, the infecting organism or cell, or (b) the anti-body (amboceptor) for the same organism or cell, either or both of which may be present in the serum.

The patient's serum, which is to be tested, is collected in the usual way, and the complement containing serum is obtained from the blood of a normal animal. Then the antigen or anti-body with which the patient's serum is to be mixed is procured in the following manner:

(a) In the first instance an emulsion of the suspected in-

fecting organism is made in 0.85% NaCl solution. The age of the growth is immaterial; but where a pure culture of the organism is not obtainable, as in syphilis, another measure is necessary. In this case (6) a syphilitic fœtus is obtained and the organs (liver, spleen, kidneys, supra-renal glands, heart, and lungs) are macerated in 0.85% NaCl solution, and placed in a cold chamber for 24 hours. At the end of this time this emulsion is filtered until the filtrate is clear. It is considered that this fluid contains the syphilitic antigen in solution. But there is one objection to this method, and that is that this antigen action is lost after six days. A much better method (11) is to put the fœtal organs in a desiccator and dry them thoroughly, when a powder may be obtained containing the syphilitic antigen, which retains its power for months. When the experiment is to be performed a small quantity of this powder is thoroughly mixed with 0.85% NaCl solution and then filtered, and the filtrate will then contain the syphilitic antigens ready for immediate use. A third method is to keep the organs in a frozen condition until required (19).

(b) The anti-bodies of the infecting organism are obtained by immunizing an animal to the special organism. Then the serum of this animal is collected in the usual manner and inactivated at 56° C. This serum should then contain the amboceptors for the immunizing organism, but no complement.

Finally the hæmolytic system should be prepared. This consists of sensitized erythrocytes, prepared by mixing together equal volumes of erythrocytes (5 per cent emulsion), and inactivated hæmolytic serum. The strength of the latter should be tested and enough added to the red cells to cause hæmolysis when complement is introduced.

These substances having all been prepared, the experiments may now be undertaken. The first step is to demonstrate the presence or absence of specific anti-bodies. This is undertaken in the following manner:

The patient's inactivated serum is diluted to varying strengths and a known quantity (0.5 cc.) is placed in each of a series of small test tubes. Then to each of these is added an equal volume of bacterial emulsion and also the same volume of normal serum containing complement (1-10), and the total placed in the incubator for half an hour. The sensitized corpuscles are now added to each tube and the mixture incubated for two hours (see Table I below). They are then placed in the ice chest over night. The tubes are finally

TABLE I.

Patient's serum inactivated at 56° C.	Bacterial emulsion.	Normal rabbit serum 1-10.	Rabbit serum immune to erythrocytes inactivated at 56° C. 1-10.	Emulsion of erythrocytes 5%.
0.5 cc. 1-10	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
0.5 cc. 1-20	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
Etc.	Etc.	Etc.	Etc.	Etc.

examined to determine the presence or absence of hæmolysis. If the former has occurred, we know that the complement was not fixed to the bacterial receptors. Therefore, no anti-bodies were present in the patient's serum. But on the other hand, if hæmolysis is not present, we infer that the complement was bound to the bacterial receptors by the anti-bodies contained in the patient's serum, or, in other words, "fixation of the complement" or "Komplementbindung" has occurred. This is regarded as then constituting a specific reaction.

If "fixation of the complement" has not been demonstrated, it is necessary to proceed with the second experiment, or that for the detection of the antigen. The method for this procedure is practically the same as the former. The only difference is the substitution of an inactivated bacteriolytic serum for the bacterial emulsion. (See Table II.) As

TABLE II.

Patient's serum inactivated at 56° C.	Animal serum immunized to special bacteria. Inactivated at 56° C. 1-10.	Normal rabbit serum.	Rabbit serum immunized to erythrocytes. Inactivated at 56° C. 1-10.	Emulsion of erythrocytes 5%.
0.5 cc. 1-10	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
0.5 cc. 1-20	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
Etc.	Etc.	Etc.	Etc.	Etc.

in the former experiment, hæmolysis or no hæmolysis will indicate a negative or a positive result. No hæmolysis signifies that the complement has been "fixed"; that is to say that it has become attached to the antigen in the patient's serum, through the medium of the immune bodies in the immune animal's serum.

In explanation of this reaction, our knowledge at the present time enables us to say that besides bacterial amboceptors, other bodies in immune serum, such as precipitins (18) have also the power to bind complement. From the diagnostic standpoint, it is immaterial which of these bodies is active, all being the products of infection or immunization.

In order to place reliance upon such experiments, it is necessary that a control be made by substituting normal human serum for that of the patient.

In the following protocols the foregoing methods have been carried out and checked by the necessary control tests.

CEREBRO-SPINAL FEVER.

CASE I.—E. H. Hospital No. 58,109, male, aged 34.

The illness began on February 13, 1907, and from this time the ordinary features of cerebro-spinal fever developed. He was admitted to the hospital on February 16, 1907. On the following day lumbar puncture was performed, and 15 cc. of turbid fluid were withdrawn. Cover glasses and cultures showed the presence of Diplococcus intracellularis. He gradually grew worse and died on February 23.

On February 18, the patient's serum was tested for the presence of amboceptors for Diplococcus intracellularis, by the method of complement fixation. The following chart (III) shows the experiments in a graphic form.

CASE II.—H. L. Hospital No. 57,780, male, white, aged 19.

The patient took ill on January 18, 1907. He was admitted to the hospital on January 19. Lumbar puncture was performed at once, and 12 cc. of fluid were removed. This was turbid and contained many pus cells with intra- and extra-cellular diplococci,

decolorizing when stained by the Gram method. Cultures proved the organisms to be Diplococcus intracellularis. The patient died on February 15.

The post-mortem examination revealed typical lesions of epidemic cerebro-spinal meningitis. On January 31 the patient's serum was tested for the presence of meningococcal anti-bodies. The result was positive, as is shown below in Chart III.

CASE III.—E. C. Hospital No. 56,460, female, aged 17.

Early on the morning of March 11, 1907, the patient had a severe rigor, with pain in the head and neck, nausea, and vomiting. These symptoms persisted to a marked degree, and she was admitted to the hospital on March 15.

CHART III. MENINGOCOCCUS MENINGITIS.

No.	Patient's serum inactivated at 56° C.	Meningococcus emulsion.	Normal rabbit's serum 1 in 10.	Rabbit's serum immune to guinea-pig corpuscles inactivated at 56° C. 1 in 10.	Guinea-pig erythrocytes 5%.	Result.
1	.....	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	Hæmolysis complete.
2	.....	.....	"	"	"	"
3	.....	.....	.....	"	"	" absent.
	Normal serum.					
4	0.5 cc. 1 in 10	.... ..	0.5 cc.	"	"	" complete.
5	" 1 " 10	0.5 cc.	"	"	"	" "
6	" 1 " 20	"	"	"	"	" "
7	" 1 " 40	"	"	"	"	" "
8	" 1 " 80	"	"	"	"	" "
9	" 1 "160	"	"	"	"	" "
	Case I.					
10	0.5 cc. 1 in 10	.....	"	"	"	" "
11	" 1 " 10	0.5 cc.	"	"	"	" absent.
12	" 1 " 20	"	"	"	"	" "
13	" 1 " 40	"	"	"	"	" "
14	" 1 " 80	"	"	"	"	" "
15	" 1 "160	"	"	"	"	" partial.
	Case II.					
16	0.5 cc. 1 in 10	.....	"	"	"	" complete.
17	" 1 " 10	0.5 cc.	"	"	"	" absent.
18	" 1 " 20	"	"	"	"	" "
19	" 1 " 40	"	"	"	"	" "
20	" 1 " 80	"	"	"	"	" "
21	" 1 "160	"	"	"	"	" partial.
	Case III.					
22	0.5 cc. 1 in 10	.....	"	"	"	" complete.
23	" 1 " 10	0.5 cc.	"	"	"	" absent.
24	" 1 " 20	"	"	"	"	" "
25	" 1 " 40	"	"	"	"	" "
26	" 1 " 80	"	"	"	"	" partial.
27	" 1 "160	"	"	"	"	" complete.

On examination the patient was drowsy, eyes partly closed, no photophobia or strabismus. A suggestion of herpes was present at the right angle of the mouth. There were no subcutaneous or submucous hæmorrhages. Temperature was 103.8 degrees Fahrenheit. The knee-jerks were absent, there was no clonus and Babinski's sign was not obtained. There was marked stiffness of the neck and Kernig's sign was positive on both sides. The white blood corpuscles numbered 22,040. On March 16, a lumbar puncture was done and 40 cc. of slightly yellowish fluid were obtained under a pressure of 390 mm. (of fluid). Microscopical examination showed the fluid to contain many cells, both mononuclear and polymorphonuclear. Lumbar puncture was repeated on March 18, with a similar result. Smears and cultures did not show any organisms. Up to this time the patient had remained in the same condition as on entrance to the hospital, but now she gradually began to improve. Kernig's sign, stiffness of the neck and the headache disappeared, and the patient was discharged on April 17.

On March 19 the patient's serum was tested for anti-bodies to the meningococcus by "fixation of complement" with a positive result. (See Chart III.)

A few explanations may be made in regard to the controls, which are of great importance in correctly carrying out the experiments.

Tubes 1 and 2 show that when the patient's serum and the organism are omitted, fixation of complement does not take place. In 3 no hæmolysis has occurred, since the tube contained no complement. Tubes 4, 10, 16, and 22 are introduced to demonstrate the fact that the absence of bacteria will allow hæmolysis to proceed in spite of the presence of inactivated human serum. The tubes 5 to 9 inclusive show that normal inactivated human serum does not contain immune bodies for the organism tested, the complement remaining unfixed, and thus allowing hæmolysis to take place.

Considering now Cases I, II and III, in which "fixation of complement"' has been demonstrated, it will be noted that positive results occur within certain varying degrees of dilution of the amboceptor containing sera, showing that the amount of complement fixing substance (amboceptor) may vary in different cases of any one disease. In these three cases it is apparent that the sera possess qualities different from those of normal serum. Plainly, the inference is, that the reaction denotes a present or recent infection with the meningococcus. Corroborative proof of this inference was in fact furnished by Cases I and II, whereby the meningococcus was obtained in culture from the fluid in the spinal canal. All results being negative in Case III, the clinical diagnosis of meningococcus meningitis was confirmed by the positive result obtained in this experiment of "fixation of complement."

CHART IV. GONORRHŒAL ARTHRITIS.

No.	Human serum inactivated at 56° C. *Normal.*	Gonococcus emulsion.	Normal rabbit serum 1 in 10.	Rabbit serum immune to guinea-pig corpuscles inactivated at 56° C. 1 in 10.	Guinea-pig corpuscles 5%.	Result.
1	.....	1 cc.	0.5 cc.	0.5 cc.	0.5 cc.	Hæmolysis complete.
2	.....	.....	"	"	"	"
3	.....	.....	.....	"	"	" absent.
4	1 cc. 1 in 10	.....	0.5 cc.	"	"	" complete.
5	" 1 " 10	1 cc.	"	"	"	" "
6	" 1 " 20	"	"	"	"	" "
7	" 1 " 40	"	"	"	"	" "
8	" 1 " 80	"	"	"	"	" "
9	" 1 "160	"	"	"	"	" "
	Case I.					
10	1 cc. 1 in 10	.....	"	"	"	" "
11	" 1 " 10	1 cc.	"	"	"	" absent.
12	" 1 " 20	"	"	"	"	" "
13	" 1 " 40	"	"	"	"	" "
14	" 1 " 80	"	"	"	"	" "
15	" 1 "160	"	"	"	"	" slight.
	Case II.					
16	1 cc. 1 in 10	.....	"	"	"	" complete.
17	" 1 " 10	1 cc.	"	"	"	" absent.
18	" 1 " 20	"	"	"	"	" "
19	" 1 " 40	"	"	"	"	" "
20	" 1 " 80	"	"	"	"	" "
21	" 1 "160	"	"	"	"	" complete.
	Case III.					
22	1 cc. 1 in 10	.....	"	"	"	" complete.
23	" 1 " 10	1 cc.	"	"	"	" complete.
24	" 1 " 20	"	"	"	"	" "
25	" 1 " 40	"	"	"	"	" "
26	" 1 " 80	"	"	"	"	" "
27	" 1 "160	"	"	"	"	" "

GONORRHEAL ARTHRITIS.

CASE I.—D. M. Medical No. 20,780, male, aged 32.

The patient contracted gonorrhœa in December, 1906, and this was followed on December 28 by an attack of acute polyarthritis. On March 21, 1907, his blood was tested for gonococcal

anti-bodies. At this time there was a chronic arthritis and also a chronic urethritis. (See Chart IV.)

Case II.—N. B. Hospital No. 57,936, male, aged 35.

The patient had had gonorrhœa six times. The last attack was in November, 1906. Early in December he developed an acute polyarthritis, involving chiefly the hips and the right ankle.

On March 21, 1907, when his blood was tested, physical examination showed a chronic arthritis, chronic urethritis, and chronic prostatitis. (See Chart IV.)

Case III.—C. B. Hospital No. 57,952, male, aged 35.

The patient had gonorrhœa in November, 1906, which was followed in January, 1907, by an acute polyarthritis. On March 21, the joint involvement was slight, but there was still a slight chronic urethritis.

On this date his blood was examined for anti-bodies by "fixation of complement." (See Chart IV.)

Chronic Gonorrhœal Urethritis and Prostatitis.

Case IV.—J. G. Dispensary No. E3107, male, aged 19.

The patient contracted his first attack of gonorrhœa three months before admission. A chronic urethral discharge continued, and on admission examination showed a chronic urethritis and a mild grade of prostatitis. The patient's blood was examined on April 3, 1907. (See Chart V below).

Case V.—J. S. Dispensary No. E133, male, aged 29.

The patient developed an acute attack of gonorrhœa in the middle of January, 1907. This was of a very severe character and persisted until admission, April 3. In February he had an acute cystitis and prostatitis. The cystitis subsided, but on admission there was still a chronic urethritis and prostatitis.

His blood was examined on April 3. (See Chart V below).

Chart V.

No.	Human serum inactivated at 56° C. *Normal.*	Gonococcus emulsion.	Normal rabbit serum 1 in 10.	Rabbit serum immune to guinea-pig corpuscles inactivated at 56° C. 1 in 10.	Guinea-pig corpuscles 5%.	Result.
1	... .	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	Hæmolysis complete.
2	.....	.....	"	"	"	"
3	....	.....	... . .	"	"	" absent.
4	1 cc. 1 in 10	.....	0.5 cc.	"	"	" complete.
5	" 1 " 10	0.5 cc.	"	"	"	" "
6	" 1 " 20	"	"	"	"	" "
7	" 1 " 40	"	"	"	"	" "
8	" 1 " 80	"	"	"	"	" "
9	" 1 "160	"	"	"	"	" "
	Case IV.					
10	1 cc. 1 in 10	..... .	"	"	"	" "
11	" 1 " 10	0.5 cc.	"	"	"	" absent.
12	" 1 " 20	"	"	"	"	" "
13	" 1 " 40	"	"	"	"	" "
14	" 1 " 80	"	"	"	"	" partial.
15	" 1 "160	"	"	"	"	" "
	Case V.					
16	1 cc. 1 in 10	.....	"	"	"	" complete.
17	" 1 " 10	0.5 cc.	"	"	"	" absent.
18	" 1 " 20	"	"	"	"	" "
19	" 1 " 40	"	"	"	"	" "
20	" 1 " 80	"	"	"	"	" "
21	" 1 "160	"	"	"	"	" partial.

The technique and the controls employed in this series of gonococcal infections were identical with those used for the cases of meningitis.

In Case I "fixation of complement" occurred when the patient's serum was diluted 1 in 80 or even 1 in 160; in Case II, 1 in 80; in Case III, 1 in 80; in Case IV, 1 in 40, and in Case V, 1 in 80.

Although the technique in these experiments is much more difficult than that in the precipitin reaction, and the results perhaps no more specific, yet the end reaction is much more definite. As Neisser and Sachs have shown "fixation of the complement" may be present in a much higher dilution of the patient's serum than that which would give a visible precipitate. It might here be urged that the difficulties of technique in this method of diagnosis almost prohibit the method for routine work. Yet in the investigation of special cases, or in the study of the etiology of certain affections, this method offers great possibilities.

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"DIVERSION OF COMPLEMENT" IN HÆMOLYSIS.

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The discovery by Pfeiffer (1) in 1895 that large amounts of immune serum did not protect an animal from the cholera vibrio, although smaller amounts were efficacious, called attention to the relationship between the components of bactericidal immune serum. During the next few years Loeffler and Abel (2), Leclainche and Morel (3) made similar observations in working with other organisms. But it was not until 1901 that an explanation of this phenomenon was sought experimentally. In this year Neisser and Wechsberg (4) demonstrated an analogous reaction in vitro and proceeded to seek the explanation. They studied the effect of a bacteriolytic immune serum when varying amounts of the inactivated serum were employed. The quantity ranged from .0005 cc. to 1 cc. To each of these amounts constant volumes of normal serum and bacterial emulsion were added. They found no bacteriolysis where large and small amounts of immune serum were used. But where medium amounts were employed, the bacteriolysis was complete.

In a second series, the amounts of the reactivating serum were varied, while the inactivated immune serum and the bacterial emulsion remained constant. The result was as in the first instance; that is, with relatively small amounts of reactivating normal serum, bacteriolysis did not occur. By further experiments, they demonstrated that inactivated normal serum was inert, and also that normal serum having slight bacteriolytic action was rendered inactive by the addition of a large quantity of inactivated immune serum. Furthermore they showed that agglutinins did not play a part in this phenomenon. Similar results were obtained when these experiments were repeated, using hæmolytic serum and erythrocytes.

From these experiments Neisser and Wechsberg conclude that where the amboceptors are in great excess, a portion combine with the complement, leaving some free, and that these free amboceptors unite with the receptors before the activated amboceptors do, and thus the complement-amboceptor group is rendered inert.

Gay (5), however, in 1905, performed experiments which seemed to contradict the above conclusions. He held that it is the precipitation (produced by the union of serum and antiserum) which causes the diversion of complement. In the experiments performed by Gay, rabbit serum immune to ox corpuscles was used. The red cells were washed very thoroughly, which he claims was not done in the Neisser-Wechsberg experiments, in order to exclude the possibility of any ox serum being present. He first produced varying degrees of precipitation by adding constant amounts of ox serum to varying amounts of inactivated immune rabbit serum. To these mixtures and to controls, in which no ox serum was present, equal amounts of normal rabbit serum and sensitized ox corpuscles were added. Hæmolysis occurred in inverse ratio to the amount of precipitate found. Where there was a marked precipitate, no hæmolysis occurred; while in those tubes in which there was no precipitate, and in the controls, hæmolysis was complete.

Thus it will be seen that the views of Neisser and Wechsberg and those of Gay are directly opposed, and both are supported by careful experimentation. It was with the object of approaching this problem from a somewhat different standpoint that the following experiments were undertaken.

A rabbit was highly immunized to guinea-pig corpuscles. The immune serum diluted 1 in 2400 caused complete hæmolysis. In order to overcome possible sources of error, the erythrocytes used were washed in 0.85 per cent sodium chloride solution *twelve* times, 15 cc. of the salt solution being used for each washing. Thus any serum attached to the corpuscles would be reduced to an infinitesimal quantity. In the collection of the immune and normal sera, the greatest care was exercised to prevent hæmolysis or contamination with any foreign material. In addition, when the substances were combined, unnecessary agitation was carefully avoided. In fact, every source of error was eliminated as carefully as possible.

EXPERIMENT I.

The rabbit serum immune to guinea-pig corpuscles was heated to 56° C. for half an hour. It was then diluted ten times, and 1.5 cc. of this dilution was added to 0.1 cc. of normal rabbit serum, which had been diluted 100 times. This mixture was incubated at 37° C. for half an hour to allow combination, and then 1.5 cc. of 5 per cent guinea-pig corpuscles in 0.85 per cent sodium chloride solution were added. The total was incubated for one hour at 37° C. At the end of this time the corpuscles were carefully precipitated by centrifugalization and the degree of hæmolysis noted. None was present.

The supernatant fluid was carefully pipetted off, and to this 0.2 cc. of normal rabbit serum (1 in 100) was added, and this mixture again incubated for half an hour. Then 1.5 cc. of guinea-pig corpuscles were added and the total incubated for one hour as before. The corpuscles were again centrifugalized and the degree of hæmolysis observed. Still there was none present.

The supernatant fluid was again treated as in the first instance, except that now 0.4 cc. of normal rabbit serum (1 in 100) was introduced. It was found there was still no hæmolysis. The procedure was repeated, employing a larger amount, 1.5 cc., of normal rabbit serum (1 in 100). When this part of the experiment was completed, it was found that total hæmolysis had occurred. This experiment was controlled in the following way. Equal quantities of inactivated

rabbit's serum immune to guinea-pig corpuscles (1 in 10) and 5 per cent emulsion of guinea-pig erythrocytes were mixed and no hæmolysis occurred; but when normal rabbit serum (1 in 5) was added in equal volume, the hæmolysis was complete. These results are shown in the following chart.

CHART I.

	Rabbit's serum immune to guinea-pig corpuscles inactivated at 56° C. 1-10.	Normal rabbit serum. 1-100.	Guinea-pig corpuscles 5%.	Time of incubation.	Result.
A	= 1.5 cc.	0.1 cc.		½ hour.	
A_1	= A.		1.5 cc.	1 "	No hæmolysis.
B	= Supernatant Fluid of A_1	0.2 cc.		½ "	
B_1	= B.		1.5 cc.	1 "	" "
C	= S. F. of B_1.	0.4 cc.		½ "	
C_1	= C.		1.5 cc.	1 "	" "
D	= S. F. of C_1.	1.5 cc.		½ "	
D_1	= D.		1.5 cc.	1 "	Complete hæmolysis.
	Controls.				
	Rabbit's serum immune to guinea-pig corpuscles, inactivated at 56° C. 1-10.	Normal rabbit serum. 1-5.			
I	1.5 cc.		1.5 cc.	1 "	No hæmolysis.
II	1.5 cc.	1.5 cc.	1.5 cc.	1 "	Complete "

EXPERIMENT II.

The corpuscles used in the three parts of the first experiment in which no hæmolysis occurred were carefully preserved. These were now repeatedly washed in 0.85 per cent sodium chloride solution until all the serum was removed. Undue shaking or any other cause of artificial hæmolysis was carefully avoided.

These corpuscles were now treated with 1.5 cc. of normal rabbit serum (1-5) and incubated for one hour. Complete hæmolysis occurred. But on the other hand the mixture of ordinary guinea-pig corpuscles (5 per cent emulsion) and normal rabbit serum (1-5), in the same amounts as before, produced no laking whatsoever. This is shown in the following chart.

CHART II.

	Corpuscles.	Normal rabbit's serum (1-5).	Time of Incubation.	Result.
I	From A_1, B_1 and C_1 of Experiment I ..... 1.5 cc.	1.5 cc.	1 hour.	Complete hæmolysis.
II	Unused corpuscles as in Experiment I ... 1.5 cc.	1.5 cc.	1 "	No hæmolysis.

EXPERIMENT III.

Inactivated rabbit serum immune to guinea-pig corpuscles (1-10) in the amount of 1.5 cc. was combined with 2.2 cc. of normal rabbit serum (1 to 100) and incubated for half an hour at 37° C. This amount of normal rabbit serum was exactly the same as the total amount used in the four parts of the first experiment. At the end of this time 1.5 cc. of guinea-pig corpuscles were added and all incubated for one hour. They then were centrifugalized as in the other experiments, and it was found that no hæmolysis had occurred. This is shown in Chart III.

CHART III.

	Rabbit's serum immune to guinea-pig corpuscles inactivated at 56° C. 1-10.	Normal rabbit serum 1-100.	Guinea-pig corpuscles 5%.	Result.
A	1.5 cc.	2.2 cc.		
A_1	= A.		1.5 cc.	No hæmolysis.
	Control.			
	1.5 cc.		1.5 cc.	" "
		2.2 cc.	1.5 cc.	" "

It will be seen from these experiments that a certain definite relationship must be reached between the amount of amboceptors and complement before hæmolysis will take place. From the first experiment, it is apparent that after the primary incubation, both activated and inactivated amboceptors are present in each of the first three parts. The inactivated amboceptors, however, seem to have the greater affinity for the receptors and, being greatly in excess, were immediately bound. This process occurred successively, until all or nearly all of the amboceptors were removed or activated. That this was brought about in two ways is made probable; first by the removal of inactivated amboceptors attached to the blood cells, and second, by the gradual addition of the complement.

The proof that the first of these events occurred is demonstrated in the second experiment, in which hæmolysis of the sensitized corpuscles occurred by the simple addition of sufficient complement. That the relatively great preponderance of amboceptors over the complement was the cause of the absence of hæmolysis is the most probable inference when the result of the third experiment is considered. In this case, as is shown, the first stage of Experiment I was repeated with the exception of one detail. The amount of complement used is equal to the total amount added in all stages of the first experiment. Yet there was no hæmolysis, while the same amount of complement had previously given a positive result. But the cause of the difference is very apparent. In the third experiment, there was a diversion of complement after the manner stated above. In the first experiment there was also diversion in the previous stages, until, by the successive removal of amboceptors by the corpuscles and the fractional addition of the complement, the relation between the complement and the amboceptors had reached the proper proportion. Then hæmolysis occurred.

That a precipitate formed by the mixture of immune serum with an homologous normal serum may cause "*fixation*" of complement has been well demonstrated by Gay, (5) Muir and Martin (6), Moreschi and others. Muir and Martin found that a visible precipitate was unnecessary for this to occur, and that even 0.00001 cc. of homologous serum would give complete "fixation." In the above experiments the great care taken to remove the guinea-pig serum from the corpuscles reduces the possible amount of serum to .0000000000001 cc.

at the most. In view of these facts, the chance of such a reaction being accountable for the phenomena observed may be disregarded.

CONCLUSIONS.

I. Receptors may have a greater affinity for inactivated than for activated amboceptors.

II. Receptors may remove amboceptors in the presence of complement without hæmolysis taking place.

III. The precipitate due to the reaction of precipitin and precipitinogen is not necessary for "diversion" of the complement.

IV. "Diversion of complement" occurs when the amboceptors are greatly in excess of the complement, and hæmolysis occurs when these substances are present in proper proportions.

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NOTE ON THE PRODUCTION OF AN AGGLUTINATING SERUM FOR BLOOD PLATELETS.

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In precipitating blood corpuscles contained in a blood-sodium citrate or blood-sodium oxalate mixture, it is found that the supernatant fluid is always opaque, while the supernatant fluid obtained after rewashing such precipitated corpuscles in sodium chloride solution is clear and transparent. To investigate the cause of this opacity, the opaque supernatant fluid was removed and again centrifugalized at a high speed for one hour. The fluid now became almost clear and a white sediment was found, which on microscopical examination was seen to consist entirely of blood platelets. By mixing this thoroughly in salt solution, a homogeneous emulsion of the platelets may be obtained.

Having obtained such an emulsion of blood platelets, free from red blood cells, it was next attempted to produce a serum containing immune bodies to these platelets. For this purpose the platelets obtained from 5 to 10 cc. of human blood were injected into the ear vein of a rabbit. Several such injections were made and the agglutinating power of the serum was tested. As it was difficult to obtain large amounts of the platelet emulsion, the tests were made by the microscopical method employed in performing the Widal test. It was found that such a serum had acquired the power of causing marked agglutination in the emulsion of platelets. Where strong emulsions are employed, the reaction is easily seen by the naked eye. In low dilutions such a serum also causes the clumps of platelets to disintegrate after a short time. It has lytic as well as agglutinating properties for platelets.

The following protocol shows the result of such an experiment, and also the fact that such a serum has no action on the platelets of an animal of the same species.

RABBIT A.

July 3. Platelets obtained from 10 cc. normal human blood injected into the marginal ear vein of a rabbit.

July 9. Injection as above.

July 19. Injection as above.

July 25. Serum tested as to agglutinating power for platelets.

	Dilution.		Results.
Rabbit immune serum.	1:10	Emulsion human platelets.	Instantaneous agglutination. Lysis after 1 hour.
Do.	1:50	Do.	Agglutination well marked.
Do.	1:100	Do.	Agglutination definite in 1 hour.
Do.	1:200	Do.	Agglutination doubtful.
Do.	1:1	Emulsion rabbit platelets.	No reaction.

It next seemed important to determine whether such a serum exhibits hæmagglutinating and hæmolytic powers, as well as agglutinating and lytic powers for platelets. The hæmagglutinating and hæmolytic powers of the serum of a normal rabbit for human red blood corpuscles was therefore tested, and it was found that it caused hæmolysis in dilution of 1:1 and slight agglutination in dilution of 1:10, but in greater dilutions was inactive. It caused absolutely no agglutination of human platelets, even when undiluted. This rabbit was then inoculated with platelets from 10 cc. of blood, and one week later its agglutinating and lytic powers were tested. It now caused marked agglutination of human platelets in dilutions of 1:20. The hæmolytic and hæmagglutinating powers were not changed.

It should be stated, however, that several more active agglutinating sera for platelets were found to have slightly more action on red blood corpuscles than has the untreated

normal rabbit serum. It is very difficult to obtain emulsions of platelets for injection, however, which do not contain an occasional red blood corpuscle. From what we know of other cytolytic sera, it is not improbable that such sera, if very active, even if produced by the injection of pure platelets, may have some action on red blood corpuscles. The above experiment, however, which was repeated several times speaks for a considerable grade of specific action.

To approach this problem in another way, we produced a hæmolytic serum by the injection into rabbits of human red blood corpuscles obtained by defibrination. The platelets are always caught in the fibrinous meshwork, and it is probable that such corpuscles have mixed with them few or no platelets. By the injection of these corpuscles into a rabbit, a serum having hæmolytic action in dilution of 1:600 was obtained. The agglutinating power for human platelets was now tested, and it was found that such a serum was entirely inactive, even in dilution of 1:1.

These experiments seem to speak quite definitely against a genetic relationship between platelets and red blood corpuscles. This question seems to have been quite definitely settled by the observations of Wright[1] on the origin of blood platelets. These experiments, however, offer additional evidence against the view of Kemp and others that platelets are derived from red blood corpuscles.

The method of obtaining platelets and the production of immune sera also obviously offers an opportunity for the study of the rôle of blood platelets in coagulation. In order to study this question satisfactorily, however, large amounts of the active sera are necessary. Preliminary experiments made by mixing such active serum with human blood and testing the variation in coagulation time so caused by means of Boggs' modification of the Brodie-Russell coagulometer have given negative results.

[1] Virchow's Archiv.

SOME OBSERVATIONS UPON BLOOD PRESSURE AND PULSE FORM.

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The Strasburger Method for the Determination of Minimal Blood Pressure—Its Inaccuracies, Their Correction, and Its Actual Value.

The value of the clinical determination of the minimal as well as of the maximal blood pressures has been so clearly demonstrated by the work of Erlanger and Hooker (1), and the later writers, Janeway (2), v. Recklinghausen (3), Sahli (4), Strasburger (5), Fellner (6), and D. Gerhardt (7), that the question of the method by which these determinations will be made becomes all important. By far the most accurate, most certain, and easiest of execution in the hospital or laboratory is the original method of Erlanger (8), made with his elaborate and rather bulky apparatus, which is otherwise almost perfect in point of manipulation. The disadvantages are (*a*) bulk and weight, and (*b*) expense, which have thus far been sufficient to keep it out of the hands of many practitioners and have prevented it from coming into use in making the routine blood pressure estimations in the wards of even the best hospitals. The bulk of the apparatus has also prevented its use in the house to house visits of practicing physicians.

The second method of determining minimal pressure, that of T. C. Janeway, consists in watching the movement of the mercury-column as the pressure in the cuff of the Riva-Rocci apparatus is lowered and in determining the point at which the oscillations due to the pulse are maximal. This method is successful mainly with pulses of large volume.

The third method, that of Sahli and Masing, consists in taking the pulse tracing at the wrist while compressing the arm above with the Riva-Rocci apparatus; pulse tracings are taken at different levels of pressure, and the points below which no further increase in the size of the pulse wave takes place are determined. In the cases in which I have controlled this method with the Erlanger apparatus, the results have been quite accurate, provided the slightest slipping of the sphygmograph was prevented or was corrected by careful readjustment; the process involves, however, a large amount of time and care, and limits the application of the method to the quietest of patients.

A fourth form of apparatus is the hæmodynamometer of Oliver, in which the movement of a bubble of stained alcohol in a closed-tube manometer is watched and the maximum of its movement selected; the error of the method is due to the varying value of 1 mm. of movement in different parts of the pressure scale.

The fallacy of the observation by Oliver's method is obviated in the sphygmoscope of Pal (9), in which the pressure on both sides of the column of alcohol is the same, except for the slight change of pressure due to the pulse wave. The Pal apparatus in its present form is, however, at least as bulky and as costly as the Erlanger and is certainly in many respects less trustworthy, although it is far from being an apparatus to be discarded.

The fifth method is that introduced by Strasburger. The pressure in the cuff upon the upper arm is gradually increased, while the observer palpates the pulse at the wrist and determines the point at which (and below which) the volume of the pulse is maximal. This method has gained great favor in Germany and upon it are based most of the more recent data of publications upon blood pressure. Its accuracy, at least in the hands of skilled observers, is vouchsafed by the tests of Brush, who found the error of careful estimations to be only 5 to 8 mm., and in my own hands frequent control with

the Erlanger apparatus entirely bears out his claims. This method has been in use in the Johns Hopkins Hospital during the past year, especially in the routine blood pressures made by the students;[1] the results of this general routine use have been far from satisfactory, so that students and internes have come to place no reliance upon it whatever. The reason for this discrepancy lies mainly in the lack of uniformity of pressure exerted by the palpating finger upon the radial artery. If it is possible for the observer to retain a perfectly uniform pressure upon the artery, the variations in the size of the pulse are readily appreciated, but if the size of the pulse under the finger is alternately decreased and increased by unsteadiness of the palpating finger, it becomes obviously impossible to determine when the pulse reaches its maximal oscillation.

It is very easy, however, to eliminate these variations in the pressure of the palpating finger by observing a simple precaution, by which this source of error may usually be eliminated even in the hands of those who have been in the habit of committing it in the highest degree. This precaution consists in simply *laying the tips of the index and middle fingers upon the lower end of the radius just lateral from the artery, and palpating the pulse with the balls instead of with the tips of the fingers.* Under these conditions variations of pressure by the fingers are exerted against the bone and not against the artery, and the main source of inaccuracy disappears.

There is, however, a further source of error not to be neglected, namely, that of inaccuracy in subjective perception. In order to minimize this it is necessary to disregard the first reading, using it merely to accustom the fingers to the changes in size which the pulse will undergo, and then to make at least five or six subsequent readings in order to eliminate the occasional errors of any single determination (which may be as high as 20 mm. Hg.). In a series of six determinations carefully made, at least four of them should give results within less than 5 mm. of each other, even when made by different individuals; and these results should be within 5 mm. of the results obtained by control with the Erlanger apparatus. Such has been my experience in the wards of the Johns Hopkins Hospital even when the determinations were made under my supervision by the very persons who had been obtaining the worst results with the Strasburger method. The pulse was felt by one person and the manometer (which he could not see) read off by another; the results were accurate if the above-mentioned precautions were taken.

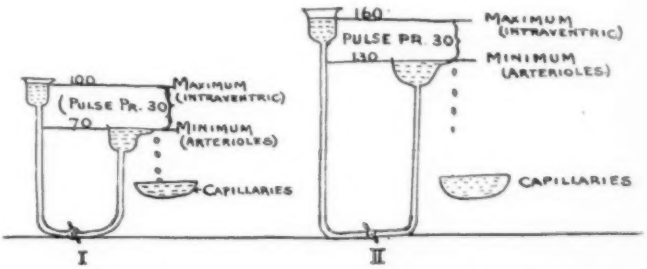
It is, therefore, clear that determinations of minimal blood pressure by the method of Strasburger may be of great value in the clinical estimation of blood pressure, particularly in the individual case in the hands of a careful practitioner; the maximal pressure may be fairly well estimated by the method of Riva-Rocci—with the broad Recklinghausen cuff. In general I have found that the readings by the method of Erlanger average about 5 mm. higher than the readings by digital palpation, though they sometimes coincide and are often 10 or even 20 mm. higher. The best results, therefore, possible for those observers who do not use the Erlanger apparatus, are obtainable by taking the minimal pressure by the method of Strasburger and the maximal by adding 5 mm. to the readings obtained by the method of Riva-Rocci (with the broad cuff). This will give in most cases results which may be quite uniformly accurate, and certainly comparable with each other, in the hands of one man, but very inaccurate in the hands of another less careful.

Determinations of this sort, therefore, though a legitimate aid to the practitioner who has reason to be certain of his own individual accuracy, should scarcely be used for published results. Unfortunately, the greater bulk of the work upon minimal pressures, especially by our German colleagues, is based upon these methods and subject to the fallacies discussed; in critical reviews of blood-pressure work the possibilities of these errors should be borne in mind.

Should the Minimal Blood Pressure be Expressed as a Fraction of the Maximal?

Another mistake of European observers consists in the expression of minimal blood pressure as a percentage of the maximal, somewhat regardless of the meaning of these quantities. This method of recording has crept into even such careful work as that of D. Gerhardt (l. c.). In general the maximal blood pressure represents approximately the intraventricular pressure, while the minimal pressure represents the algebraic sum of all the factors of resistance to outflow through the arterioles, viz., viscosity of the blood, arterial friction, breadth of bed, total-arteriole cross-section at the given instant. It is clear that the main resistance occurs not in the capillaries but in the arterioles which are most subject to the action of the vasomotors. As Dawson has shown, the minimal pressure is practically constant throughout the arterial system, at least as far as the saphenous artery in the dog, while the maximal falls gradually as the arteries become smaller; hence, at the very smallest arterioles, when maximal and minimal probably closely approximate each other we find both about equal to the minimal pressure in the aorta.[2]

The pulse pressure (the difference between maximal pressure and minimal pressure) then represents the difference between the greatest pressure in the aorta and the pressure in the arterioles; or in other words, represents the force tend-



[1] The pressure was gradually diminished instead of being gradually increased.

[2] The limits of experimentation prevent an accurate knowledge of the maximal and minimal pressures in arteries smaller than the saphenous of the dog, but these relations probably hold true in the main down to much smaller arteries.

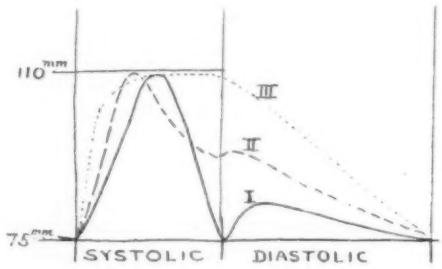
ing to drive the blood towards the periphery. The conditions at once become clear when we think of them in the light of the following experiment upon the model (Fig. 1). Let us suppose the maximal pressure (intraventricular pressure) is represented by a reservoir 100 cm. above the base or zero level, and that the minimal pressure is represented by a second reservoir (approximately the pressure in the smallest arteries and arterioles) only 70 cm. above the base, the two reservoirs being connected by a long rubber tube descending between them to the base zero, and closed by a revolving stop-cock. It is evident that the force driving liquid from maximal to minimal reservoir is equal to only 30 mm. of water, and the minimal reservoir must overflow before liquid flows out of it to a larger reservoir at a lower level arranged to catch such overflow (capillaries and veins). In this case the minimal pressure would be represented by 70 per cent of the maximal. Suppose the two reservoirs were raised to 160 and 130 cm., respectively, above the base. Their relations to one another would be unchanged, liquid would flow from one to the other at the same rate and hence would flow onward at the same rate to the venous system, but the percentage relation would here be quite different, being raised to 80 per cent or exactly what it would have been if instead of both being raised (the one from 100 to 160, the other from 70 to 130) the maximal pressure had fallen to 87 cm. and the outflow had diminished. Moreover, as has been shown by the experiments of Erlanger, Dawson, and Henderson, the pulse pressure is the correct measure of the volume of output of the heart under physiological conditions.

The absolute figures for the maximal and minimal pressures are of importance, but their relations expressed in percentages of the maximal pressure are very misleading. For example, with a maximal pressure of 220 mm. Hg. (from a case of chronic nephritis) a minimal pressure of 160 mm. would be expressed as 70 per cent, and would, therefore, be associated in significance with a maximum of 110 mm. and a minimal of 77 mm.—perfectly normal figures. Obviously the simple figures mean much more than the percentage, and the use of the latter should be discouraged.

The Dicrotic Pulse in Typhoid Fever.

Another point closely related to the study of the maximal and minimal pressures, particularly from the standpoint of Sahli's absolute sphygmogram (l. c.)[3] is the relation of pulse form to maximal and minimal blood pressures. This point has also been investigated by D. Gerhardt (l. c.), and especially by H. A. Stewart (10) in this laboratory. As the most interesting features and discussion of this problem will appear in the publication of the latter, it will suffice to mention here only the conditions present in the dicrotic pulse of typhoid fever. While serving as House Officer in the Johns Hopkins Hospital in 1903 to 1904, it was not infrequent for me to find either in the same patient at different stages of the disease or in different patients, maximal pressures of 110 to 115 mm. with minimal pressures of 75 to 80 mm. (determined with the Erlanger apparatus) both when the pulse was dicrotic and when the dicrotism had disappeared. The dicrotic pulse always corresponds with an extremely low dicrotic notch. In other words, as the dicrotic notch marks the end of the systolic period in the pulse (Marey (11), Huerthle (12)), the very low dicrotic notch indicates that the pressures in the aorta and in the more peripheral vessels have almost equalized themselves before the end of the period of systolic output, which is possible only in conditions of marked peripheral dilatation. Other signs of peripheral dilatation that clinically go hand in hand with the dicrotic pulse are the flushed face, the "bounding pulse," with rapid filling and rapid depletion of the arteries.

There are, of course, three forms of pulse curve possible, with the same maximal and minimal pressures (Fig 2):



I. In which the fall of the pulse is almost complete by the end of systole. This is the form of the dicrotic pulse.

II. That in which only about half the fall occurs during systole. This is the pulse of the normal individual.

III. The anacrotic pulse in which the rise is continuous throughout systole and the fall begins at the beginning of diastole.

It is clear from the foregoing that the first type represents the condition in peripheral dilatation and the last represents that in peripheral constriction, and this is further borne out by the work of Stewart. Evidently, therefore, the dicrotic pulse corresponds to the condition of maximal dilatation of both peripheral and abdominal vessels (the latter always present in typhoid) and yet nevertheless the maximal and minimal blood pressure is the same as in the second group of typhoid cases in which the dicrotic pulse is not present. Now it is evident that when there is peripheral vasodilation, the total arterial reservoir is increased and it is not unlikely also that the diameter of the aorta and larger vessels also is widened. (Since vasomotor nerves are present in the larger vessels; and since the carotid and other vessels of the dog may in the course of an experiment be seen to change their caliber by at least one-third, similar changes in the larger vessels in man are most probable.) But in order to fill a larger arterial bed with blood to the same head of pressure and to maintain the same pressure in diastole as in a smaller arterial bed, it is evident that a greater systolic output is necessary, and accord-

[3] The pulse curve plotted upon coordinate paper with the ordinate of maximal pressure at the summit of the curve, and the ordinate of minimal pressure just before the beginning of the next pulse wave.

ingly in cases with dicrotic pulses we usually have other evidences of increased heart action, ringing aortic second sound and strong cardiac pulsation—a fact to which my attention was called by Dr. Barker.

It might appear at first sight that the conception that the cardiac output with the dicrotic pulse is greater than is necessarily the case in the same patient after subsidence of dicrotism (and diminution of vasodilation) is at variance with the rules of Erlanger (l. c.), Dawson (13), and Henderson (14) above cited, that the pulse pressure is proportional to the systolic output. This rule is dependent upon the assumption that the diameter of the aorta is constant, which is no doubt the case in most normal individuals or in those where the vasomotor system is in tolerable equilibrium, but it certainly does not apply in all pathological conditions, especially where extreme peripheral dilatation is present. (The greatest discrepancy is found in experimental aortic insufficiency in which the pulse pressures may be multiplied at least four times with scarcely any change whatever in cardiac output, measured by Henderson's plethysmograph). (Stewart l. c.)

It, therefore, seems most in accord with the facts to conclude that the dicrotic pulse of typhoid fever is the result of the coincidence of very marked peripheral dilatation with somewhat increased heart action, and that it may disappear with the subsidence of either of these factors.

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SOME VARIATIONS IN THE FORM OF THE VENOUS PULSE.

A PRELIMINARY REPORT.

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Since the publications of James Mackenzie (1) have made the forms of the venous pulse well known, there has been little tendency among writers upon the subject to differ as to the form of the physiological venous pulse, although quite recently Bard (2), of Geneva, has published a series of tracings from normal individuals which do not coincide exactly with those of the former. Bard finds present in his tracings a small wave intermediate between the presystolic (auricular) wave and the carotid wave of the venous pulse, and concludes that this wave is due to the systole of the ventricle in the interval before the aortic valves have been opened. I have frequently noticed in my own tracings that the so-called (*c*) wave ascribed by Mackenzie to the transmitted pulse from the carotid occurred at an appreciable interval before the carotid pulse (as much as .05 sec. or about the time necessary to open the aortic valves) and can fully agree in Bard's interpretation,[1] as against Mackenzie's. Mackenzie's view has, moreover, been absolutely refuted by the beautiful tracings obtained by Morrow (3) upon animals, in which the carotid has been ligated both centrally and peripherally without diminishing the size of the (*c*) wave, and it is further borne out by the exactly similar tracings obtained from within the auricle itself. I have never, however, been able to obtain tracings resembling those of Bard in which this systolic wave appeared not merely

[1] In fact I had given the same explanation of this wave in a paper read before the Johns Hopkins Medical Society on February 19, 1906, and published in the American Journal of Medical Sciences in September, 1906, a little later than Bard's paper.

as the beginning of the slope to the carotid wave, but quite distinct from it. Bard, moreover, finds a similar splitting of the postsystolic or (*v*) wave (whose crest is marked by the opening of the tricuspid valve) into two varying waves which he designates by the letters (*d*) and (*f*). Undulations upon the surface of the (*v*) wave are not infrequent but are far from being the rule, and the tracings published by Bard, although representing a different, and perhaps better, technique than usually employed do not accord well in these regards with the best tracings by other observers.

following the *v* wave, and at the end of the subsequent rapid filling of the vein, and gives rise to a small elevation closely resembling that produced by an auricular contraction, providing the latter were not followed by systole of the ventricle. This condition might well be present providing there were disturbances of conductivity, but in neither of the cases in which the phenomenon has been observed were any other signs of disturbed conductivity present; the conduction time (*a-c*) interval was normal and there was no bradycardia. It is true that frequently the conductivity for an auricular ex-

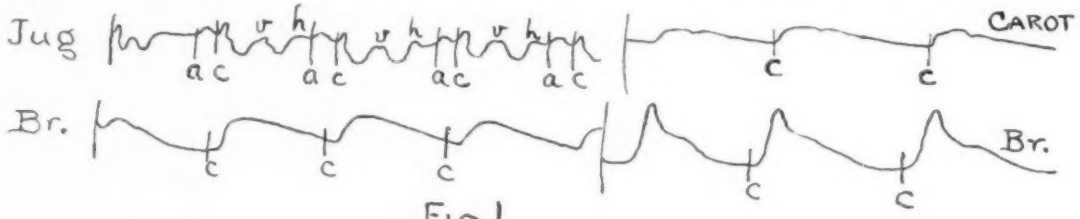

Fig 1.

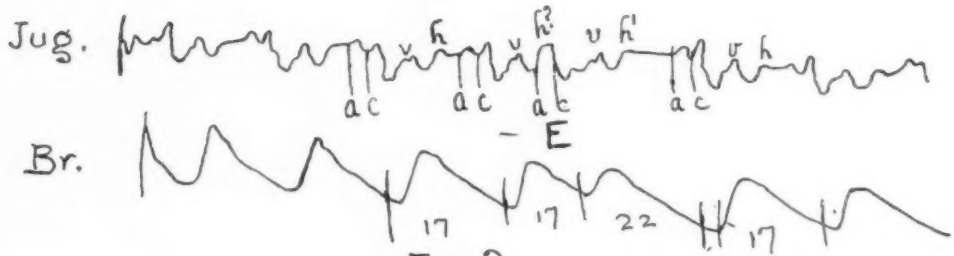

Fig 2.

Fig 3.

FIG. 1.—*Jug* = pulse from jugular vein; *br* = pulse from brachial artery; *carot* = pulse from carotid artery; *a* = auricular presystolic wave; *c* = time of occurrence of wave in carotid artery; *v* = postsystolic wave, the crest of which marks opening of tricuspid valve; *h* = wave during diastole; from a patient with myocardial disease.

FIG. 2.—From same patient after exercise, showing extrasystole at *E*, probably auricular in origin. Lettering as in Fig. 1, Arabic numerals refer to space in mm. between successive pulse waves in brachial pulse.

FIG. 3.—(After Morrow.) Tracing from jugular vein of a dog, showing shoulder (*h*) upon curve during diastole, corresponding to wave (*h*) in human venous pulse. Only a part of Dr. Morrow's figure is reproduced.

From the above mentioned facts it is evident that great variations occur in the form of the venous pulse of different individuals, although in the main they adhere quite closely to the conventional type as described by Mackenzie. Recently, however, I have occasionally encountered a slight variation of form occurring in the diastolic portion of the cardiac cycle, which might well be confounded with disturbances in conduction or with auricular extrasystoles, and which therefore merits attention.

The wave referred to (Fig. 1, *h*) occurs after the collapse

trasystole is less than that for the regular beats, but as shown by Hirschfelder and Eyster (4) this applies only for early extrasystoles occurring well within the first third of the cardiac cycle, and does not apply to the period when the wave under consideration is produced. This reasoning justified exclusion of the auricular extrasystole in the first case in which this unusual wave was encountered, and in which the question of disturbed conduction was one of great importance to the patient. That it could not indicate a true partial block was

shown by the fact that the wave was not exactly midway between the two definite auricular contractions.

On another occasion, after vigorous exercise an extrasystole took place at exactly the time of the wave (Fig. 2). This extrasystole was followed by ventricular contraction and after the extrasystole the same wave Fig. 2 (*h*) occurred in exactly the same portion of diastole, without any relation to the auricular waves, and without further disturbance of the cardiac rhythm. This in itself, as well as the subsequent occurrence of the same wave in a perfectly healthy individual with perfectly normal heart seems sufficient to justify the conclusion that it is to be regarded as an event in the normal diastole, more pronounced in some hearts than in others.

It will be noticed that in the very excellent tracing obtained by Morrow (3) from the jugular vein of a dog (reproduced below, Fig. 3,) that just at this point in diastole there is a very suggestive elbow upon the pulse tracing (*h*), and this point corresponds to the end of the most rapid stage of filling of the veins after the ventricle has been filled. This joint corresponds more or less to the time at which the cardiac plethysmograph shows that the main filling of the ventricle has occurred (Henderson), and at the time when, according to Henderson (5), model experiments show that the mitral and tricuspid valves are closed by the floating together of the cusps. Whether or not this wave is actually produced by the floating together of the cusps, and whether such a forcible undulation may be responsible for the third sound of protodiastolic gallop rhythm cannot at present be stated.[2] In the first case observed, the heart sounds were extremely distant, and no note of the gallop rhythm was made during his entire stay in the hospital. In the second case, the heart sounds were loud and clear, but at the end of expiration a very faint third sound coinciding with the time noted in protodiastolic gallop rhythm was audible in the tricuspid area, at the time the venous pulse was taken. This sound was not loud enough, however, to be definite, and none of the tracings which I have made at other times upon cases with gallop rhythm have shown this peculiar variation; so that as yet no definite conclusions as to the relations of this unusual wave of the venous pulse to either the closure of the tricuspid valve or the gallop rhythm are warrantable.

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THE RAPID FORMATION OF ENDOCARDITIC "VEGETATIONS."

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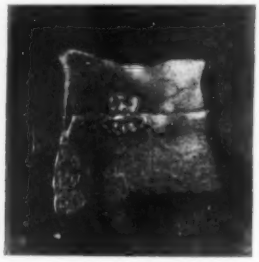
The cauliflower-like form assumed by the vegetations of verrucose endocarditis has always been a matter of some interest and curiosity to both pathologist and clinician, and it has been customary to assume that the unevennesses in these warty outgrowths were brought about by the more or less unequal contraction of the organizing fibrin along the strands of Zahn. Undoubtedly this mechanism does play a large and important rôle in the formation of endocarditic vegetations, but, in some cases at least, the form of the latter may already be determined in the thrombotic deposit which collects upon the valve immediately after the production of the lesion. This became most strikingly apparent in the experiments upon artificial aortic insufficiency in the dog, performed by Dr. H. A. Stewart and myself during the past winter. The lesions of the valve were all produced either by means of a blunt button-pointed probe or by a MacCallum valvulotome introduced through the left carotid and thrust through the aortic valve. In every case the heart responded, after a few beats, by a marked fall in the minimal blood pressure, with little or no change in the maximal;[1] and the character of the pulse became collapsing. In a few of the experiments it was observed that about an hour later the minimal blood pressure again rose to near normal, and the collapsing character of the pulse disappeared. At autopsy in these cases it was found that the puncture through the valve had become plugged with fresh yellow fibrin and blood-platelets, which in one instance had assumed definite cauliflower-like outlines (as shown in the figure). This "vegetation"—really a thrombotic deposit—was absolutely fresh and non-adherent; the lesion to the valve

[2] Since the above has gone to press Theobald (Deutsches Arch. f. klin. Med., Leipz., 1907, XC, p. 85, Fig. 13) has published a veinous tracing from a dog, showing a definite *h* wave after vagus stimulation. On the other hand, experiments with dead hearts have thrown some doubt upon Henderson's view that the auriculo-ventricular valves close during diastole.

[1] Stewart, H. A. An Experimental and Clinical Study Upon the Pulse and Blood Pressure in Aortic Regurgitation. Thesis, University of Edinburgh, 1907. To be published later.

had been produced only one hour before and there was absolutely no sign of previous lesion. The mass itself consisted of



"Vegetation" of Fresh Fibrin One Hour After Puncture of Aortic Valve.

a soft homogeneous yellow thrombus and, on handling even gently disintegrated like any other *intra vitam* clot, leaving no trace of organized material. The interesting features connected with it are two-fold: First, the rapid formation of plugs in the freshly formed holes in the valves, sufficiently firm temporarily to repair the damage. Such a rapid temporary repair may play a large part in the recovery from spontaneous rupture of an aortic valve after over-exertion, and perhaps also in staying the course of the leak in acute endocarditis. Secondly, since the soft fibrinous vegetation in the course of its formation had already assumed the verrucose form, this could not be the result of contraction of strands of tissue, but the fibrin was probably deposited in the stalk-like forms by the to and fro current of the blood passing through the valve, other irregularities being most likely the result of eddy currents. In the permanent vegetation this form would of course be modified largely by the subsequent contraction of the "organizing fibrin," although the resemblances of these fresh fibrinous deposits to the usual form of vegetation found in aortic insufficiency renders it possible that this modification of form is not as great as might be supposed.

THE CALCIUM, MAGNESIUM, PHOSPHORUS, AND NITROGEN METABOLISM AND THE ORGANIC ACIDITY OF THE URINE IN A CASE OF SO-CALLED PHOSPHATIC DIABETES, WITH COMMENTS UPON AN INTERESTING FORM OF ACIDOSIS.

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In 1876 L. J. Teissier described a number of cases which had much in common from a clinical standpoint. The principal symptoms were polyuria, very acid urine of high specific gravity and increased excretion of phosphates in the urine. Some of the patients suffered from nervous symptoms. In another group of his cases glycosuria with all the typical symptoms of diabetes developed, and cataracts were often found. For this latter reason Teissier thought it justifiable to call this symptom-complex "*phosphaturie à forme diabétique.*" By doing so he created a new form, "essential phosphaturia," which he distinguished from phosphaturia in the sense known at that time, viz., the excretion of turbid urine due to the precipitation of phosphates. Later Ralfe, in the London Lancet of 1887, referring to the original article of Teissier, confirmed the findings of the latter by describing a few more cases of this kind. On going over the literature of phosphorus metabolism we have found this "phosphatic diabetes" mentioned frequently by investigators.

Recently Dr. H. Greenbaum, of Baltimore, sent one of his private patients into the Medical Clinic at the Johns Hopkins Hospital with symptoms resembling very closely those described by L. J. Teissier as characteristic of phosphatic diabetes.

The patient, a Jewess, 39 years of age, complained of headache, occasional stiffness below the right knee, itching of the skin, dryness and cracking of the skin, hyperacusis, constipation, susceptibility to colds and increased frequency of urination.

The family history is negative except on the nervous side. Several members of the family have been excitable. One uncle died in an insane asylum and one cousin was also insane. The patient's father was a nervous man, dying at 63 of some form of paralysis.

As a child she had measles, but remembers no other illnesses. She married 21 years ago. About 12 years ago the patient lost some 12 pounds in weight in the course of a short time, suffered from cracking of the heels and from boils on the body and from infected finger tips. She consulted Dr. Osler, who found the urine of high specific gravity but it contained no sugar. Her appetite was ravenous and her thirst marked. She complained of sinking spells, during which she would become dizzy and things would look black to her, though she did not fall. She suffered from great weakness. She often felt chilly when others were quite comfortable. As the patient was going to Europe, Dr. Osler referred her to Dr. von Noorden, who in turn sent her to Carlsbad under

the care of Dr. Kraus. There the patient took the waters (Carlsbader sprudel) and was given electrical treatment. She remained in Carlsbad for six weeks and later spent three weeks in St. Moritz, where she improved generally. The boils disappeared and the heels were better.

The patient has given birth to ten children and in addition has had four miscarriages, though no evidence of lues in her or her family could be found. The living children are all healthy. She has had some local gynæcological lesions which have been treated surgically. At the menstrual period she has headache, pains in the legs, is nervous, irritable, and tends to lose control.

During the past twelve years the patient has had periods of improvement alternating with periods when the symptoms were prominent. A year ago she suffered much from cardialgia. When her symptoms are worse the itching is troublesome, especially under the chin, on the chest and under the breasts. The skin often becomes hyperæmic at these times. When her symptoms are marked the frequency of urination is increased. During the past three months the patient has noticed falling of the hair. A feeling of constant pressure on the top of the head is complained of. She sleeps very poorly and tires easily.

The appetite is good. There is no cough or dyspnœa. She wears glasses prescribed by Dr. Friedenwald for her "eye-strain."

On examination the woman was found to have a large frame and to be rather over-nourished, weighing 177 pounds. The skin is pale and the lips slightly cyanotic. Tongue clean. No jaundice. Pupils active. Eye-muscle movements good. Pulse 21 to the quarter, regular in force and rhythm. Radial just palpable. Epitrochlears not enlarged. No enlargement of the lymph glands of the neck. Heart and lungs normal. Lower pole of right kidney palpable; left not felt. Sigmoid palpable. Abdomen otherwise negative. Knee-kicks active and equal. No clonus. No œdema of the ankles. No relaxation of the sacro-iliac joints. The blood was normal.[1]

The urine on examination showed high specific gravity (1030) in spite of the polyuria (2-2½ l.) very great acidity (1500), remarkably large output of nitrogen (27 gms.) and P_2O_5 (5.7 gms.); uric acid normal (0.4 gms.).

As a careful metabolic study of such a case has never been made before we thought it interesting to undertake this work. The patient came to the hospital for eight days and was put for the whole period on the Folin diet, a purin-free food consisting of 119 gms. of protein, 225 gms. of carbohydrates and 148 gms. of fat. It was greatly to our advantage to use this diet, for Folin in his admirable investigation of the composition of normal urines employed it in order to set up normal standards for the urinary constituents. Comparisons, therefore, with the figures he obtained, facilitate the explanation of pathological findings. The water intake was kept constant for the entire period, a factor which, of course, has an influence on the urinary excretion. The experiment was divided into three periods:

Period I preceded the time when the patient came to the hospital. The urine of a number of days was saved (for each 24 hours) at the patient's home and sent the same day to the laboratory for examination. It was not possible during this period satisfactorily to control the patient's food. This was however accomplished during the eight days of the second period, spent in the hospital. (*Period II*). In *Period III* the patient was at her home again and the total amount of urine was collected as in *Period I* for a few days.

From the findings in the patient's urines previous to admission to the hospital it was clear along which lines the metabolism experiment should be directed. There was a hyperacidity of the urine with high phosphoric acid excretion. The question presented itself, to what is this increase in acidity due? Is it dependent on the presence of large amounts of acid salts, or to an increase in the organic acids? The possibility of having to deal with free mineral acids was ruled out *a priori;* they never occur free in the urine. Besides the acidity of the urine, the total nitrogen (method of Kjeldahl) the ammonia (Folin), the P_2O_5 (Volhard), the calcium oxide and magnesium oxide (gravimetrically by precipitation as calcium oxalate and magnesium ammonium phosphate, respectively) were determined.

The organic acids (free and in the form of the neutral salts) were carefully estimated. In regard to the method used for the organic acids it may not be out of place to say a few words of explanation. Magnus-Levy in his investigations on the amount of organic acids in the urine of diabetic individuals used a very complicated method which was until a few years ago the only available one for this purpose. It consists in determining separately on one side all the inorganic bases (potassium, sodium, ammonia, calcium, magnesium), on the other side all the inorganic acids (HCl, H_2SO_4 and H_3PO_4). Both are reckoned in terms of acidimetric units. By subtracting the value of the bases from the value of the acids there is always found a small excess of bases which can be due only to the presence of organic acids. These organic acids are combined with the excess of inorganic bases. Under normal circumstances uric acid and hippuric acid are probably the most important organic acids. Magnus-Levy's method does not take into account any organic acids, which might be present in the urine as free acids and not in the form of neutral salts. Such a possibility is not at all ruled out by any of the work done on the acidity of the urine. Folin is very

[1] The patient was also studied at one time by Dr. Thomas R. Brown of Baltimore, who found a high acidity of her urine. Dr. Brown mentions this case in his article on "Urinary Hyperacidity, a Consideration of Cases with Symptoms Suggestive of Cystitis, but with no Infection due to this Cause." In this interesting paper, the author describes over ten cases, all neurasthenic women. The urine, when tested for the acidity gave always extraordinary high results. As soon as the patients were put on a rest cure and alkalies were given with the food, the symptoms disappeared rapidly. Brown warns against treating such patients for cystitis, as deplorable results may follow. He does not attempt to explain the high acidity of the urine, but is rather in favor of the view that the cause does not lie in the diet and that some pathological condition of the nervous system is responsible for the hyperacidity.

much in favor of admitting the presence of free organic acids in certain pathological urines. As the organic acids are very weak acids, the inorganic bases will combine first with the stronger inorganic acids (sulphuric, hydrochloric, phosphoric acid), and only if there be an excess of bases, are these latter used to neutralize the organic acids. The method used by us in the course of the present investigation was described

	Food.				Urine.														Feces.			
	N. in gms.	P_2O_5	CaO.	MgO.	Vol. Sp. gr.	N.	NH_3 ratio.	P_2O_5 mineral.	P_2O_5 org.	CaO.	MgO.	Fixed Alk. in cc. $\frac{N}{10}$	Total Acidity.	Org. Acids in cc. $\frac{N}{10}$	Weight in gms.	N.	P_2O_5.	CaO.	MgO.
Dec. 1.....					2000	27.2		5.71											
" 3.....					1050	31.2		3.31		.33		−.962							
" 5.....					2250	34.6		3.23		.765		−.459							
" 8.....					2125	19.8		2.85		.711		+.54							
" 16.....	17.6	3.75	2.63	.348	3000	16.4	3.7	2.71	.40	.864	.100	−.378	595	593	19	.91	.55	.19	.08
" 17.....	17.6	3.75	2.63	.348	1650	15.3	3.8	3.30	.25	.593	.110	−.201	754	982	35	1.37	.83	.49	.17
" 18.....	16.9	3.84	2.64	.349	1740	16.6	4.0	3.05	.09	.347	.201	−.226	748	974	26	.50	.64	3.88	.09
" 19.....	16.9	3.84	2.64	.349	1450	16.1	4.1	3.07	.40	.594	.157	−.315	689	847	11	.50	.34	10.08	.07
" 20.....	15.2	3.87	1.81	.179	1650	17.8	6.0	2.02	.25	.358	.177	−.163	697	1393	97	4.23	3.15	.16	.08
" 21.....	15.2	3.87	1.81	.179	1800	14.6	5.1	2.88	.17	.398	.101	−.587	684	691	13	.57	.38	1.04	.09
" 22.....	16.1	2.97	2.14	.258	1770	14.9	4.9	2.96	.09	.249	.147	−.400	655	779	66	3.48	1.76	1.64	.59
" 23.....	16.1	2.97	2.14	.258	1850	14.9	5.1	2.67	.02	.676	.197	−.87	664	393	44	1.61	1.12	2.55	.50
Jan. 29.....					2300	15.3	5.8	2.95				−.149	621	1107					
Mar. 6.....					1540	19.3	1.4	1.98				+.523	108	825					
" 12.....					2000	17.4	2.6	2.23				+.668	220	1224					
" 26.....					1595	8.4	11.2	2.03				−.300	468	727					
Apr. 16.....					1850	10.5	4.3					+.293	382	942					

by Folin in 1903 and modified by E. S. Edie and E. Whitley in 1906. The last two authors do not mention Folin's work and appear to regard the method as a new one. The procedure is as follows:

A. A portion of the fresh urine is titrated after saturation with crystallized potassium oxalate against the decinormal sodium hydrate solution, phenolphthalein being used as an indicator.

B. In a second portion of urine the ammonia is determined by Folin's second method.

C. A third portion is evaporated to dryness in a platinum crucible and incinerated, care being taken not to heat the crucible to more than a dull red heat (to avoid loss of sodium and potassium). By this incineration all the organic acids with the organic basic constituents of the urine as well as the ammonia are volatilized, leaving behind only the inorganic constituents. By dissolving the residue in water and titrating in exactly the same way as was done with the fresh urine one finds the inorganic acidity (leaving NH_3 out of account), that is to say, the bases liberated by volatilizing the organic acid formerly combined with them. In burning the urine the ammonia, which is one of the inorganic bases, is removed. A separate estimation of this base becomes therefore necessary. By adding this value to the alkalies measured after incineration we obtain the actual mineral acidity. This latter is subtracted from the total acidity A and gives the organic acidity, i. e., the organic acids present in the urine as salts and in the free state. For all details and theoretical discussion of urinary acidity we refer to Folin, Hoeber, Edie, and Whitley. This method yields reliable results when every precaution is taken. The use of this method in a number of other investigations carried out in the laboratory here always proved satisfactory.

	Nitrogen in gms.	Total P_2O_5.	CaO.	MgO.	Remarks.
Dec. 16..	+0.33	+0.49	+1.75	+0.162	
" 17..	+0.97	−0.47	+0.99	+0.65	
" 18..	+0.66	+0.14	−1.59	+0.52	
" 19..	+0.30	+0.41	−7.77	+0.139	
" 20..	−6.80	−2.29	+1.29	−0.079	Patient gets 3 tablets of thyroid extract.
" 21..	±0	+0.60	−0.47	−0.078	
" 22..	−2.2	−1.72	+0.26	−0.486	
" 23..	−0.4	−0.84	−1.08	−0.446	Patient gets $CaCO_3$ gr. xxx, ad doses IV (8 gms.).
Total ...	−6.14	−2.75	−6.62	−0.67	

Discussion of Results.

Phosphorus metabolism.—Teissier and Ralfe determined the phosphates in their cases as calcium and magnesium phosphates respectively. The figures obtained are not at all a true index of the phosphoric acid present because of the varying composition of the precipitates. Another point is that the phosphorus in the food and feces was not taken into consideration. It is only from a study of the complete balances, i. e., the content of phosphorus in the food, as well as in the urine and feces that we can hope to find out any irregularities in the metabolism of this element. From a number of very thorough investigations we know that phosphorus is retained by the organism more eagerly than any other substance. The reason becomes evident when one thinks of the wide distribution of the phosphorus in the human body, as a constituent of bone in inorganic combination, and as a component of the cell nucleus, and of the blood. The phosphates of the blood are of the highest importance for the maintenance of the

alkalinity of the blood. From experiments made by Walter on dogs and recently by Fitz and Alsberg on rabbits, it is known that the ingestion of mineral acids produces increased output of phosphoric acid in the urine. In severe cases of diabetes the phosphoric acid excretion is found to be increased. Administration of alkalies seems to prevent the loss. All this suggests that a production of acids within the body or their administration might result in an increased elimination of phosphoric acid. There is good evidence that the disodium phosphate of the blood gives up one atom of sodium to neutralize the acid produced; as a consequence the proportion of the different constituents of the blood is changed and the organism tries to reestablish the normal equilibrium by excreting the excess of acid phosphates formed. One would expect, therefore, to get high amounts of urinary phosphoric acids in pathological conditions in which an acidosis occurs. The urine of the days of Period I contained an exceptionally high output of phosphoric acid. The patient was very nervous during this period and all the symptoms were pronounced. Unfortunately, on beginning the complete study of metabolism in Period II the patient improved so rapidly that nothing unusual was found in the output of total phosphates. The slight retention of phosphorus observed could perhaps be explained by the composition of the food, which contained a rather high percentage of this substance, or by an attempt on the part of the organism to replace the possible loss during the period preceding the experiment.

A remarkable feature is the relatively high percentage of organic phosphorus. Symmers found that in normal urine the amount of organic phosphorus never exceeds 2.5 to 4 per cent of the total phosphoric acid. On the first day of Period II the organic phosphorus is as high as 15 per cent of the total. It decreases then rapidly and only on the fourth and fifth day is there again an increase to be noted. Neither thyreoid feeding nor the administration of calcium carbonate seems to influence the output of phosphoric acid. Of course we realize that the time of observation was very short and that inferences must be cautiously drawn. In the period after the patient had left the hospital the phosphorus excretion was very much diminished, probably as a result of the administration of alkalies during this period.

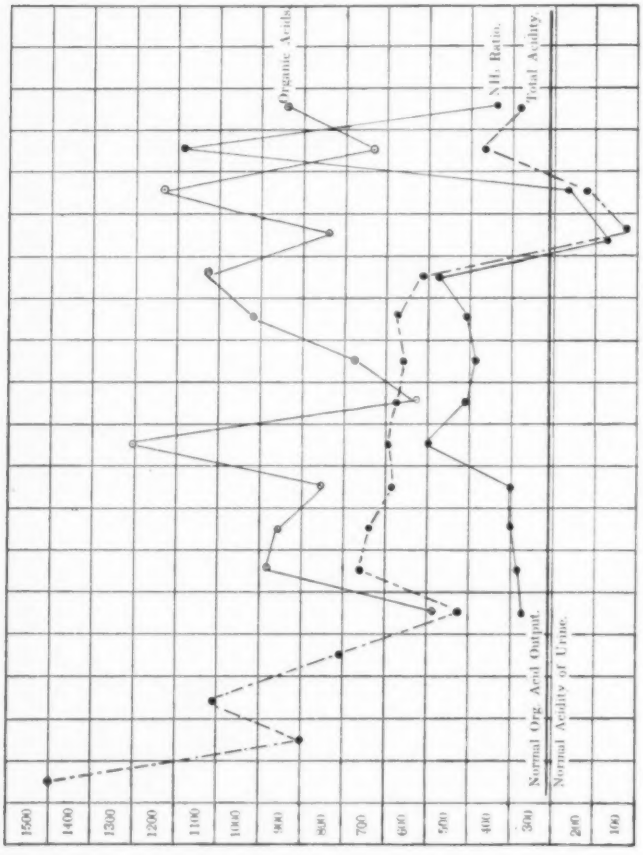
Nitrogen metabolism.—In Period I the nitrogen-excretion by the urine is extremely high, maximum 31.2 gms. We do not think that this can be due altogether to a correspondingly high intake in the food. As the patient is always losing weight during the time when the symptoms become more pronounced this high nitrogen-excretion might partially be accounted for by the breaking down of tissue. In Period II the patient was practically in nitrogen-equilibrium during the first four days. The thyreoid tablets produced a marked increase of nitrogen in the urine for one day only. The loss of nitrogen in the days following the thyreoid administration was very slight. Nothing abnormal can be noticed in the nitrogen excretion of Period III, except the relatively low excretion on March 26, a day when the patient's general condition was worse. This may be explained by the partial loss of appetite that sometimes accompanies deterioration of the patient's general condition.

Calcium metabolism.—Large amounts of calcium oxide characterized the analyses of the urines of Period I, the maximum being 0.76 gm., whereas normally not more than 0.5 gm. is excreted daily on a mixed diet. At the beginning of Period II the calcium oxide is still high, 0.58 gm. being 21 per cent of the calcium oxide in the food. Voit gives the percentage of calcium oxide in normal urine as 10 per cent of that in the food. In regard to the distribution of calcium oxide in the urine and feces during this period it may be said that 88 per cent was found in the feces and 12 per cent in the urine. This indicates either that there is a large excretion of calcium through the intestinal wall or the calcium of the food is not dissolved to any great extent. As the absolute amount of calcium oxide in the urine in comparison with the calcium content of the food is large, one would be inclined to think that the excretion of calcium through the intestine might be the cause of the large amount of calcium oxide in the feces. Our knowledge of pathological, as well as of physiological calcium metabolism, is still very incomplete. It is only in recent years that thorough studies of calcium balances have been carried out, and additional work is required first of all to establish standards under normals conditions, as they would be of great help in interpreting the findings in pathological cases.

Magnesium metabolism.—Nothing abnormal was detected in the metabolism of magnesium. Of the magnesium found to be excreted in our case 50 per cent passed through the kidney and 50 per cent through the intestines.

The acidity of the urine.—By consulting the chart illustrating the changes in the amount of organic acids, the total acidity and the ammonia ratio it will be seen that all of them show great deviations from the normal. The total acidity in Period I was remarkably high, from 1500 to 800 cc., the normal value being 300. In Period II no great changes occurred, the average value being about 700, being still more than twice the normal. After January 29, alkalies in the form of sodium bicarbonate were given to the patient and the effect was pronounced. The total acidity decreased within one day so as to be even below the normal. In spite of the administration of alkalies by the mouth, however, the total acidity showed a strong tendency to rise after March 1, and at this time the patient's condition was becoming gradually worse. On April 16 the total acidity was again low, apparently the result of a rest cure taken by the patient in Atlantic City. The organic acids in the urine increased to about 3 or 4 times the normal quantity. Unfortunately the urine of Period I was not examined for organic acids, but there is sufficient evidence from the results of the second and third period to point to a highly increased output of organic acids in that period also. In regard to the acidity another point of interest lies in the ammonia ratio. Some days the latter was very much increased (11 per cent), on other days the ratio was quite normal. After administration of alkali the ammonia is replaced by the alkalies and the ammonia ratio becomes therefore very much

diminished. The question whether the absolute amount of ammonia in the urine or the percentage of the total nitrogen represented by ammonia-nitrogen should be regarded as the index of acidosis is still open to discussion. Friedrich Müller is in favor of the former as a diagnostic measure. Schittenhelm and Katzenstein in a series of 12 experiments came to the conclusion that the ammonia ratio gives a truer picture of the degree of acidosis. In our opinion both factors may be of value. In a pronounced acidosis, as in diabetes where clinically all the symptoms point to acid intoxication one will always find a high output of ammonia in the urine, amounts of two or more grams. The ammonia determination



by itself will remove any doubt in such cases, but in a beginning acute or in a chronic acidosis the ammonia ratio may yield a more helpful result. Thus, for example, should the nitrogen metabolism be very low, as a result of fasting or of an abnormal diet containing only a little protein, the total nitrogen excretion may be much lower than that of a normal person and the ammonia be within normal limits; an acidosis would not be detected by an ammonia determination; if we were to determine the ammonia ratio in such a case, however, we would find out immediately that the latter is largely increased as contrasted with the normal and we should be perfectly justified in saying that an acidosis existed.

We may conclude, therefore, from the data obtained in the course of this investigation that in the case under consideration we had to deal with urinary symptoms that are certainly due to a pathological metabolism which manifests itself in the excretion of a relatively large amount of organic acids in the urine, accompanied by a hyperacidity, an increased excretion of organic phosphorus compounds and a somewhat abnormal calcium metabolism. The results suggest that we had to deal also with a pathological increase in the phosphates in our case, as Teissier asserts for his cases of a similar type, but we have not found sufficient evidence to establish firmly this supposition.

What was the cause of this acidosis? A short review of the knowledge of acidosis, or as it is sometimes called, acid intoxication, may help us to answer this question. Acidosis can be produced in normal individuals by a change in diet or by starvation. It is well known that on putting a man or an animal on a diet free from carbohydrates, acetonuria develops in the course of a few days. The same is true in simple fasting or starvation. The cause of the production of acetone, which is derived primarily from diacetic acid and β-oxybutyric acid has been found to be the restriction of carbohydrates in the food. It seems that carbohydrates play an important rôle in the oxidation of intermediary products of metabolism, especially those of the fats. It was Rosenfeld and Hirschfeld's fundamental investigation that demonstrated this relation. A clearer insight into the cause of diabetic acidosis was derived from the work of these authors. In diabetes as a result of a specific morbid condition the carbohydrates are not at all (or in milder cases only to a limited extent) available for the oxidation of these intermediary products of the fats (and possibly of the nitrogen-free carbon chains of the deamidized amino-acids which result from protein-cleavage), and, therefore, the urine contains acetone, diacetic acid and sometimes β-oxybutyric acid.

In many other diseases, e. g., in typhoid, scarlatina, pulmonary tuberculosis, carcinoma, in diseases of the stomach and intestines, epilepsy, eclampsia of pregnancy, diacetic and β-oxybutyric acid are sometimes present in the urine. In most of the reports on the subjects no attention is paid to the diet of the patient. It was v. Noorden who first pointed out the fact that insufficient ingestion of food may be the cause of acidosis in febrile diseases. As a matter of fact, acetone is never found in the early stages of febrile disease while the diet is still normal and there has been no lack of carbohydrate-ingestion. Further careful work must decide whether or not an abnormal metabolism is the cause of the appearance of these acids in the diseases above mentioned.

In many other morbid states diacetic acid and β-oxybutyric acid are occasionally met with in the urine. Becker described a toxic acetonuria due to disintegration of albumin and lack of oxygen, the latter being attributed to the destruction of red blood corpuscles. As an example he mentions poisoning with carbon monoxide. Recently Friedrich Mueller in a lecture on an obscure form of acidosis and acid diathesis, delivered at the Baltimore Clinical Society, stated that often in the urine

of patients presenting a sediment of uric acid crystals, he could by the method of Magnus-Levy for the determination of organic acids in the urine demonstrate that, in spite of the acid reaction of the urine, a large excess of inorganic bases was present, a finding which he could explain only by assuming the presence of an excess of some organic acid. No acetone or diacetic acid or lactic acid could be detected in these urines.

Coming back to the consideration of our case it looks as if the findings of Friedrich Mueller corresponded in many respects with ours. We were never able to demonstrate the presence of acetone, diacetic acid, or lactic acid in the urine. Furthermore, the patient's tolerance for carbohydrates was tested by her family physician, Dr. Greenbaum, who has kindly turned over to us the results of his test. The patient was given 150 gms. of sugar and the urine examined on the two following days for sugar. No reducing substance could be found. The possibility that we had to deal in this case with a disturbed carbohydrate metabolism is therefore ruled out. Millon's reagent yielded a slightly positive reaction in all the specimens examined. But tyrosin or any other oxyphenyl-containing acid alone cannot account for the considerable increase of the acidity observed. Another investigation will have for its purpose the isolation of the acids concerned, and we hope later to be able to gain more accurate information regarding their nature and source. The suspicion that the thyreoid gland could be involved was ruled out by the normal action of the patient after thyreoid feeding.

The question presents itself: Could this increased excretion of organic acids explain any of the patient's symptoms? It is not impossible that even such a comparatively moderate increase in the acidity of the urine might cause a decrease in the blood or tissue alkalinity by washing out valuable alkalies from the organism. The removal of calcium seems to have a special influence on the nervous system. Sabbatani in studying the biological action of calcium salts on the nervous system found out that the small amounts of calcium contained in nerve tissue has a moderating influence on the sensibility of the nerves. Removal of calcium salts from the nerve cells increases their irritability to such an extent that convulsions can be produced resembling those observed in epilepsy. Ronconi, a pupil of Sabbatini, as a practical application of the findings of his teacher, used subcutaneous injections of calcium bromide in the treatment of epileptic attacks.

Quest analyzed seven brains, two of them fœtal, five of them brains of children varying in age from one month to eight years; he noticed that the calcium content of the brain of the fetus and new-born child is strikingly high in comparison with that of the brains of older children. The examination of two brains of children dead of tetanus revealed a very low calcium content. The studies of W. H. Howell and of Jacques Loeb on the necessity of an equilibrium among the cations of the blood and tissue juices are very interesting. At least there are indications that calcium plays an important rôle in the functions of the nervous system. This could perhaps explain the fact that whenever an acidosis is marked the nervous system becomes more or less involved. It is fascinating as an hypothesis to think of some of the functional neuroses and psychoses, hitherto spoken of as being "without organic basis" as maladies which ultimately may be proven to depend upon "an inorganic basis," viz., a disturbance in mineral metabolism.

Conclusions.

In a case of so-called phosphatic diabetes, the study of metabolism revealed the following facts:

1. The patient excreted a urine of very high acidity, due to the presence of abnormally large amounts of organic acids, the exact nature of which has not been determined thus far, though the latter point is still under investigation.

2. The patient's carbohydrate metabolism was found to be normal, so that the existence of a larvate diabetes mellitus is ruled out.

3. Whether the appearance of the organic acids in the urine is due to (a) an overproduction compared with the normal amount, or to (b) a lack of oxidation of these intermediate products of metabolism, or whether both factors are concerned, we do not know. It may be asserted, however, that the increased output of organic acids in the urine certainly depletes the body of alkali. It is probable that the cases described by T. R. Brown would, if similarly investigated, present a similar metabolism. It seems probable, too, that a number of the cases with the same clinical symptoms described by Teissier and Ralfe would have presented similar features in regard to acidosis, had they been examined therefor.

4. Whether it is that the abnormal symptoms on the part of the nervous system presented by the patient are the result of acidosis or that the metabolism of the individual is so disturbed by the pathological action of the nervous system, as to tend secondarily to an increased excretion of organic acids, cannot be decided at present. One fact that seems to us well established by both clinical observation and metabolic study is that acidosis is closely connected with nervous disturbances.

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A STUDY OF THE METABOLISM IN A CASE OF SO-CALLED "ARTHRITIS DEFORMANS."

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The object of the investigation, the results of which are included in this paper, was to study, under the influence of a diet the constituents of which could be accurately known and controlled, the pathology of so-called "arthritis deformans" from a chemical standpoint.

Literature.—The history of the development of our chemical knowledge concerning the morbid processes in arthritis deformans is so closely associated with the subject of acidosis that the problem of a review of the literature of the former simply resolves itself into an epitome of the knowledge extant on the latter subject.

In 1880 Hallervorden (1) first observed an increase in the ammonia in urine in cases of diabetes. He concluded that in diabetes there was an overproduction of acids, to neutralize which more ammonia than usual was produced, and thus the abnormal acids were carried out of the body. Furthermore, he regarded the acids as organic in nature.

Walter (2) thought that the acids were inorganic in nature.

Stadelmann (3) succeeded in isolating an acid from the urine of a diabetic. It acted like an organic acid, and he thought that it was identical with crotonic acid.

Minkowski (4) and Külz (5) first fully pointed out the nature of this acid, as they demonstrated that the acid in question was identical with β-oxybutyric acid, and that the croton acid is accordingly not preformed in the urine, but is a secondary product.

This cleared up to a great degree the mystery of diabetic coma, and the view that this coma is an acid intoxication is largely due to the work of Stadelmann, Minkowski, and Magnus-Levy.

Among the various forms of acidosis mentioned in the literature are:

(1) Diabetic acidosis.
(2) Acidosis in children.
(3) Pathological, non-diabetic acidoses of von Noorden.
(4) Acidosis in arthritis deformans.

(1) The literature of diabetic acidosis is very extensive and does not fall within the province of this paper.

(2) There are many conditions in children which are closely associated with an acidosis, conspicuous among them being intestinal disorders of various kinds.

Keller (6) in 1896 observed an increased elimination of ammonia in the urine of children suffering from stomach-intestinal diseases. In some cases the ammonia went as high as 50 per cent.

Bergh (7) is believed to have shown that the increase in ammonia in such conditions was referable to an acidosis, since by giving alkalies he could reduce the elimination of ammonia to a minimum.

Czerny (8) observed that the respiration curves of children who died from chronic wasting diseases had a marked similarity to those of animals poisoned with acids.

The Breslau school pointed out that there was a direct relation between the amount of fat taken in with the diet and the amount of ammonia put out in the urine. Czerny and Keller thought that the fat was the source of the organic acids, and that, in the exhaustion of the infant organism, there arose unknown, abnormal acids which the body in its pathological state was unable to oxidize, as it normally would oxidize acids arising from the fats.

Steinitz (9) considered that the ammonia was used to neutralize the abnormal acids, in order to protect the body alkalies, to avoid the condition of poverty in alaklies, designated by him as alkalopenia. In a case of enterogenous catarrh of the stomach he (10) found a hyperacidity caused by the pyocyaneus bacillus, which possesses the power of breaking down the higher to lower fatty acids.

Rosenfeld (11) noted that a decrease in carbohydrates in the diet causes the appearance of fatty acids and their derivatives in the organism.

An interesting fact in connection with acidosis in children is that the acetone in the breath exceeds that in the excreta by 19 to 60 times.

(3) v. Noorden (13) gives a variety of non-diabetic conditions in which acidosis has been demonstrated.

(*a*) Febrile acetonuria, as in scarlatina, typhoid, pulmonary tuberculosis, etc.

(*b*) Carcinomatous acetonuria by no means rare.

(c) Gastro-intestinal acetonuria particularly in acute diseases of stomach and intestines (14).

(d) Puerperal eclampsia and the eclampsia of pregnancy.

(e) A very uncertain group not yet firmly established; asthma acetonium (15), certain psychoses that Wagner has designated as acetone autointoxication, and certain spasmodic states, described by von Jacksch (16) as epilepsia acetonica. Finally, a disease picture occurring in children, some cases of which have recently been described by Babinsky, characterized by spasms with excretion of acetone. The influence of a diminished diet, especially in carbohydrates, has not been sufficiently considered to place this group on a firm foundation as a clinical entity.

(f) Toxic acetonuria, after poisoning with drugs such as antipyrine, morphine, lead, chloroform, narcosis, etc.

(g) Starvation and inanition acetonurias.

(4) Very little exists in the literature on the subject of acidosis in arthritis deformans. Helen Baldwin (17) in a study of twenty-one different cases of arthritis deformans found that in all cases of rheumatoid arthritis in which the disease was progressing there was evidence of perverted metabolism, in the nature of an increase in organic acids in the urine. In three cases the stomach contents were examined, and in all there was an absence of free HCl after a test meal. The organic acidity seemed to vary with the intensity of the disease, being greater in severer than in mild cases. Evidences of intestinal putrefaction were found in all the cases in which the disease was active.

Herter (18) found a considerable excess of organic acids in many instances of dilatation of the stomach, and arthritis deformans. Furthermore, he points out that in well-developed intoxications ammonia is the chief base withdrawn from the body, but where the process is very extensive sodium and potassium, and even calcium and magnesium may be removed.

The subject of alkalinity of the body is so intimately connected with the problem of acidosis that it will perhaps be not out of place to consider very briefly the metabolism of calcium, magnesium, and phosphorus.

(1) *Calcium metabolism.*—Calcium oxide is increased in fever, Bencke (19) and Senator (20). Bencke also found the calcium elimination very much increased in chronic wasting diseases, as pthisis and carcinoma.

In diabetes v. Noorden, also Gerhart and Schlessinger, found an increased elimination.

In arterial sclerosis Hirschberg (21) in 1818 found a decreased elimination of calcium.

In arthritis deformans v. Noorden (22) twice found a retention of calcium. Moraczewski (23) found a great loss of calcium in pernicious anæmia.

Calcium is very important for the nervous system. Sabatani showed that the irritability of the brain (cerebellum) is to a large extent dependent on this chemical body.

Magnesium metabolism.—Very little is known of the part played by magnesium in the various problems of metabolism. It is much more easily eliminated by the kidneys than is calcium, and its place of absorption is the small intestine. In starvation (Cetti) the proportion of magnesium to calcium is altered, so that less of the former is gotten rid of by the body.

In arthritis deformans v. Noorden (22) found in two cases a retention of magnesium.

Phosphorus metabolism.—Normally only about 6 to 11 per cent of the phosphorus appears in the stools. In chronic malnutrition the elimination of phosphorus decreases. A diminished elimination is claimed by some authors in fever, and also in nephritis; in the latter case explained by them as due to the lessened permeability of the kidney to potassium salts. An increased elimination of phosphates was found after administration of doses of thyroid gland to a dog, E. Ross (24). Again, W. Scholz (25) has drawn attention to a connection between the function of the thyroid gland and the phosphorus elimination, pointing out that a decrease in its function causes a retention of phosphates, and an increase in its function a corresponding increased breaking down of phosphorous compounds in the body, and consequently an increased output. He draws the analogy of phosphorus standing in the intermediary metabolism to the thyroid gland as does iron to the spleen.

Von Noorden (32) found in three cases of arthritis deformans a retention of phosphorus in each case.

A consideration of the organic phosphates is given later, under division of phosphates.

The patient, Miss B., was a private patient of Dr. Barker's, who presented well-marked signs of the so-called "chronic infectious type" of arthritis deformans. She was assigned to us by him for a metabolic study. The clinical history is given here:

Patient was an unmarried woman, 32 years of age, of frail physique, and weighing on admission only 97 pounds. She complained of swollen and stiff joints.

P. H.—As a young girl patient had had measles, whooping-cough, and chicken-pox, and at this period of life suffered a good deal from catarrh of nose and head. She had not been especially subject to attacks of sore throat. At the age of 15 she had a tumor of the breast removed and there has been no recurrence. The patient has always had a capricious appetite and a tendency to constipation. She began menstruating at the age of 14. Periods have always been painful. During an attack of arthritis the year previous to admission the patient had a cessation of periods for six months.

Habits.—The patient has always led an out-of-door life and lived under the best of hygienic surroundings.

P. I.—The disease began insidiously about four years ago, and has pursued a progressive course until last summer, when the general condition improved somewhat. The patient first noticed that the knuckles began to enlarge gradually and she was unable to get her rings on. They were swollen, but not red or painful. From this, as a beginning, the disease progressed slowly, involving the wrists of both hands, the shoulders, both knees and ankles, giving a typical picture of the polyarthritic type of arthritis deformans chronica. The general nutrition suffered greatly in the course of the disease, the patient loosing 20 pounds.

P. E.—On examination a fairly general glandular enlargement was made out. The clinical findings in the various joints above enumerated showed a typical picture of arthritis deformans chronica, the joints of the hand and wrists being most characteristic. The interossei of the hands were markedly atrophied and the right knee was considerably swollen.

A soft, systolic murmur which was transmitted into the axilla, was made out at the heart apex.

A note by Dr. Rosenheim showed that there was no disease of any of the nasal accessory sinuses. Signs of chronic inflammation in a remnant of the left tonsil were made out distinctly. The patient's right knee was opened by Dr. Sowers and cultures taken remained sterile. Her "opsonic index" to streptococcus, taken by Dr. Meakins, was found to be low.

The patient was put on a known diet, the diet recommended by Otto Folin, the constituents of which are given below. The urine and fæces in 24-hour specimens were collected separately and analyzed for the various constituents given in the tables. Especial care was taken to get the urine in as well preserved a state as possible, and every precaution was taken against analytical errors from bacterial fermentation. The analyses were done, with but few exceptions, everywhere in duplicate, these exceptions being only where the amount of urine, etc., was not sufficient to permit of this procedure.

The period of observation covered five days on the Folin diet, and for three days subsequent to this a few of the constituents of especial interest were followed.

Composition of Folin's Diet.

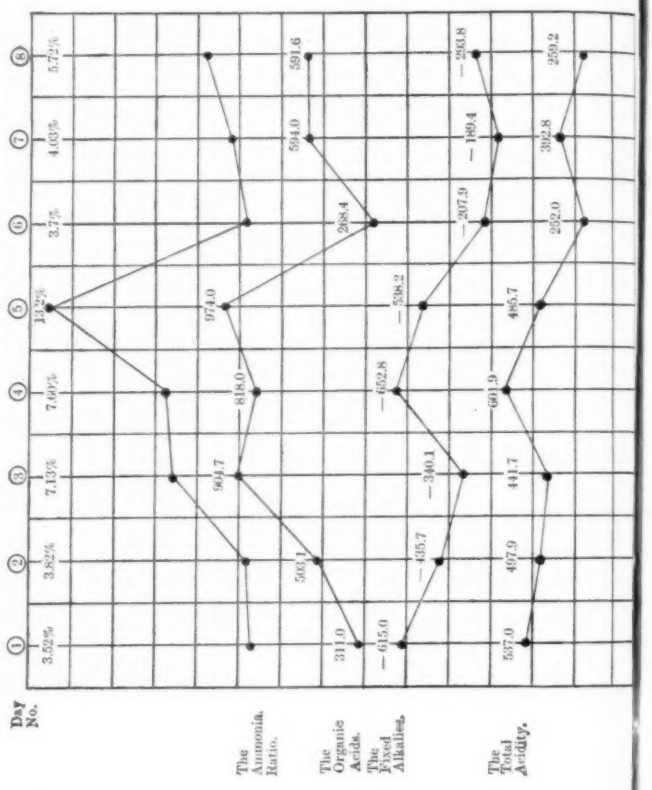
Whole milk	500 cc.
Cream (18 to 25 per cent)	300 cc.
Eggs (white and yolk)	450 gms.
Horlick's malted food	200 gms.
Sugar	20 gms.
Sod. chloride	6 gms.
Water enough to make up	2 litres.
Extra water to drink	900 cc.

This diet was analyzed for the various constituents given in the tables. It was made up in four-litre portions, so that one analysis did for two days nutrition. This diet was found to be a rather hard one for patients to take for any length of time, five or six days being about as long as it could be continued on an average. However, as Folin's valuable studies giving such a variety of urinary data in normal persons are based on this food as a diet, it was deemed advisable to make use of it also in this study of a pathological condition, in order that it might be possible to have a normal standard for comparison. Furthermore, the diet contains ample protein, carbohydrates, and fats, so that the results obtained on such a diet cannot be vitiated by a deficiency in any one of these three important constituents. Proteins, 119 gms., carbohydrates 225 gms., fats 148 gms. During the course of the investigation the patient took exactly the whole amount of food in each 24 hours and exactly the amount of the water allowed, and no drugs were given during this period. The stools were identified by the method of administering charcoal at the beginning and end of the experiment, and were carefully collected, preserved from putrefaction, and dried with addition of hydrochloric acid to prevent loss of ammonia, and powdered.

Folin's diet was calculated for its protein, etc., constituents on the basis of an individual weighing 70 kgms., which corresponds very closely with the value demanded by Voit for an individual of the same weight. As our patient weighed only 35.6 kilo. it will be seen that the diet was very liberal.

Consideration of the Analytical Data.

(1) *The acidity.*—From a consideration of the analytical results, it is quite suggestive that we are dealing in this pathological condition with an acidosis, organic in type, and mild in nature. By consulting the tables it is seen that there is a periodical increase in the organic acids which is most marked on the third, fourth, and fifth days. It will also be seen that coincident with this increase in organic acids and running parallel to it, there is an increase in the ammonia coefficient (i. e. $\frac{\text{ammo.}}{\text{T.N.}}$ ratio), which reaches its highest point 13.2 per cent when the organic acidity is highest—974.0 ccs. $\frac{N}{10}$ NaOH.



In considering the fixed alkalies and earths later, it will be observed that the ammonia is the main factor in combating the acidosis, which in this case is not of such a magnitude as to draw heavily on the reserve force of fixed alkalies and earths that the body needs so essentially for the maintenance of its vital processes. From a perusal of the literature it seems quite apparent that the damage done to the organism in acidosis is not from any inherent property of any special acid, but rather to a diminished alkalescence of the body, produced by the toxic influence of the unknown bodies, characterized by acid properties. So in diabetic coma, which is characterized by an enormous increase in organic acids, it is not any exclusive property of β-oxybutyric acid that gives it such a toxic influence, but rather that it decreases the alkalinity of the blood and diminishes the body's reserve of alkalies, which are indispensable to life.

The inquiry into the causative factor of acidosis has been

directed mainly along the lines of studies of inorganic acids, but the lower fatty and aromatic acids have begun to be studied without as yet very definite results. In this case a test for a number of the simpler acids was made. In none of the urines was there found any trace of acetone, diacetic acid or β-oxybutyric acid. In the aromatic group the test for oxyphenol was positive in every case.

The fixed alkalies.—From a study of the curve representing the elimination of this constituent in the urine it is seen that the fixed alkalies are decreased, varying from $615.0 \frac{N}{10}$ NaOH to 340.7. The elimination is in a general way parallel to that of the organic acids, being at its minimum when the organic acids are largest in amount. It is also roughly parallel to the ammonia ratio, being decreased most when the ammonia ratio is highest. From a review of the proportionate relation in these two elements in the body's chemical mechanism of defense, it would seem that they are both instrumental in combating the acidosis, but by far the greater part is performed by the ammonia.

Phosphates.—A study of the distribution of phosphates is of some interest. In the urinary fraction there is a slight increase in the total and inorganic phosphates on the days when the organic acidity is high, but the most suggestive and interesting finding is in the behavior of the organic phosphates. They are increased, reaching a maximum 0.497 gms. on the day that the organic acidity is highest. The curve of the organic phosphates is roughly parallel to that of the organic acidity.

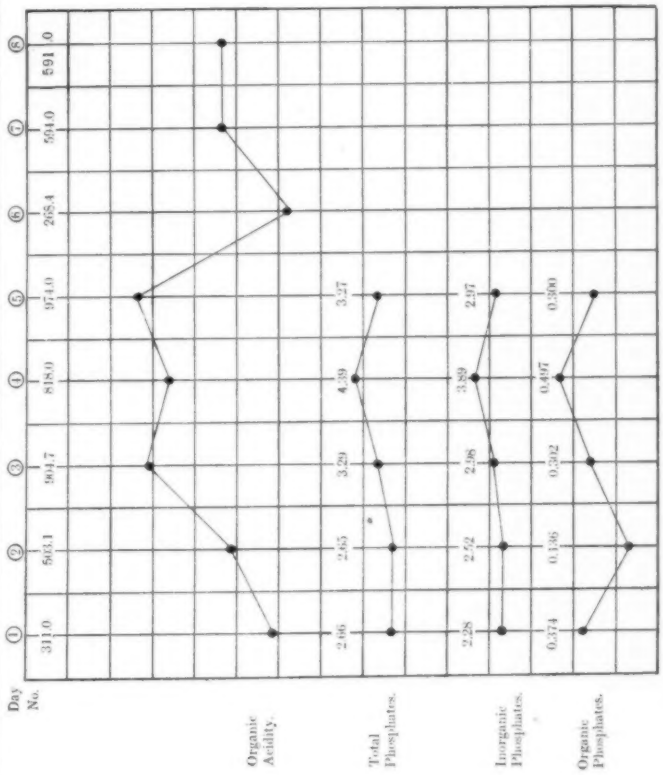
In a normal person the organic phosphates have been found not to exceed .12 gms. in 24 hours (Symmers) and represents from about 2.5 to 4 per cent of the total phosphorus excreted in the urine. In this case of arthritis deformans the amount is considerably larger, varying from 0.136 as a minimum to 0.497 as a maximum, or in percentage of the total phosphorus as high as 14.1 per cent.

The organic phosphates are probably present in the urine as glycero-phosphoric acid, or the more complex compound, lecithin. The latter, on boiling with baryta water yields fatty acids, glycero-phosphoric acid and cholin. So it is not unnatural to suppose that there might be some intimate connection between acidosis and the quantitative elimination of organic phosphates. It would be of great interest to know the cause of the increased elimination of organic phosphates in this case, but as yet little that is positive can be said regarding their appearance in the urine. Symmers (26) has found an increase in this urinary component in lymphatic leukæmia, and nervous diseases of the degenerate type. It has been suggested that the diet might have an influence on the elimination of organic phosphates, but Levy, Mandel, and Oertel (27) have shown that it is practically independent of the diet. So this factor can be apparently safely excluded. It is furthermore unlikely, as Symmers pointed out, that the increase in organic phosphates comes from the bones. Rather it is to be explained as an increase in the organic component of the endogenous phosphorous compounds, or as one of the resultant factors of a lessened power of oxidation on the part of the body.

The table showing the relations discussed above, as well as the curves, showing the relation of the organic acidity to the phosphates are given below.

TABLE I.—ORGANIC PHOSPHORUS.

Day No.	Total P_2O_5 in urine.	Organic P_2O_5 in urine.	Percentage of organic P_2O_5.
I.	2.660	0.374	14.1
II.	2.650	0.136	5.1
III.	3.290	0.302	9.2
IV.	4.390	0.493	11.3
V.	3.270	0.300	9.2
Average.	3.252	0.321	9.8



There was found, as can be seen by consulting the table of balances given, a retention of phosphorus during the entire period of observation, which was least at the height of the organic acidity. This might be expected, as it is well known that there is no mineral constituent which the body holds back as energetically as phosphorus (Albu-Neuberg (28)), and when the phosphorus is increased in the diet, the body does not at once come into phosphorus equilibrium, but retains part of the increased amount. As this is the case, here, where the patient has changed from a mixed diet relatively poor in the salts P., Ca., and Mg. to a diet richer in these components, part of the retention may be simply due to an increased supply; but it may also be advanced that in a disease of the nature of arthritis deformans where there is a waste

of tissues, the body may take a larger proportion of these salts to repair the damage effected by the morbid process.

Calcium and Magnesium.

(1) *Calcium.*—The output in the urine was very constant, varying from 0.88907 gms. to 0.69444 gms., and did not markedly increase with the organic acidity as found by Gerhardt and Schlesinger. It must be remembered, however, that the acidosis in this case is of far less extensive a type than that usually manifested in diabetes.

The amount of calcium in the fæces is largely in excess of that in the urine, varying from 1.05677 to 2.7390 gms. The curves giving the fractional distribution in the urine and fæces as well are given below. From an inspection of these, it is seen that the percentage of calcium in the urine is slightly increased, the figures given by v. Noorden being in the urine from 3.9—20 per cent and in the fæces 71.2 to 96.1 per cent.

In a study of the calcium and magnesium balance in three cases of arthritis deformans v. Noorden and K. Belgard found a retention of calcium (1.28 gms. and 0.75 gms.) and magnesium (.06 gms. and 0.34 gms.) respectively in two cases, and a loss of each in a third. Phosphorus was retained in all three. In this case of arthritis deformans a retention throughout the entire study was observed, varying from 2.10423 gms. on the first day to 0.44135 on the fifth day, or an average of 1.923 gms. Here again as in the case of the phosphorous metabolism, the retention may be due in part to an abnormal richness of the diet in calcium, and in part to conditions caused by the morbid processes of this disease.

Table II.—Calcium as CaO.

Day No.	Calcium in urine.	Calcium in fæces.	Percentage in urine of Calcium output.	Percentage in fæces of Calcium output.
I.	0.88500	1.05677	45.6	54.4
II.	0.72101	2.58650	21.8	78.2
III.	0.88907	2.27390	28.1	71.9
IV.	0.82842	1.72790	32.4	67.6
V.	0.69444	2.11220	24.7	75.3
Average.	0.80359	1.95145	30.5	69.5

(2) *Magnesium.*—The elimination of magnesium in the urine is practically constant, varying from 0.15978 gms. to .10744 gms. It is not increased with the organic acidosis, and evidently does not take a part in the neutralization of the abnormal acids, at least not to any appreciable extent. The percentage in the urine and fæces is about normal, agreeing very closely with the figures given by Bertram and Reuvall, 29-38 per cent in the urine and 67-71 per cent in the fæces. There is a retention of magnesium during the entire period of the study, with the exception of the third day, as shown in the table of balances.

Table III.—Table of MgO.

Day No.	MgO in urine.	MgO in fæces.	Percentage of MgO in urine.	Percentage of MgO in fæces.
I.	0.15978	0.15672	50.5	49.5
II.	0.11390	0.27249	29.5	70.5
III.	0.10744	0.25548	26.9	73.1
IV.	0.11110	0.17357	38.7	61.3
V.	0.11312	0.22910	33.3	66.7
Average.	0.12107	0.21747	35.8	64.2

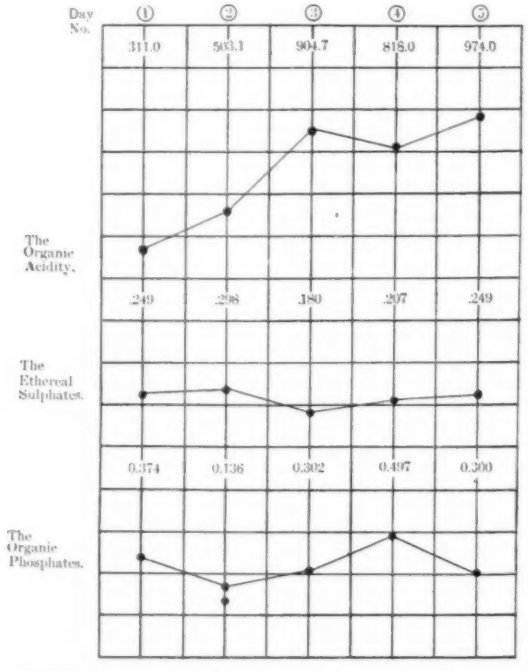
Table IV.—Table of Balances.

Day No.	I.	II.	III.	IV.	V.	Average.	Total.
Total N	+0.073	+1.11	+1.39	+0.88	+2.35	+1.16	+5.80
Total P_2O_5	+2.330	+2.170	+1.854	+0.850	+1.909	+1.837	+9.192
Total Cl	+2.110	+1.131	+0.754	+0.305	−0.048	+0.850	+4.252
CaO	+3.104	+1.738	+1.639	+1.691	+1.442	+1.923	+1.92
MgO	+0.0710	+0.0012	−0.017	+0.082	+0.025	+0.032	+0.162

Uric acid and kreatinin.—The uric acid on this practically purin-free diet is reduced to the so-called endogenous fraction, and is fairly constant, with the exception of the fourth day, when a rise occurs which is coincident with the greatest rise in the organic phosphates, suggesting that on this day there may be taking place an increased destruction of nucleins.

The study of the elimination of kreatinin on a purin-free diet, in the case under our observation, discloses, as did those of Folin, that this factor is practically a constant one and apparently independent of the changes in the total nitrogen. The absolute value of the kreatinin, however, is low in these observations, being about ½-⅓ the amount found by Folin in normal individuals, but as Folin pointed out, this low value can be explained by the fact that our patient weighed only 35.6 kgms., whereas in his observation one patient weighing 87 kgms. gave a kreatinin value of 1.6 gms. and another weighing 87 kgms., a value of 1.15 gms. The findings noted above would appear to be in accord with Folin's contention that on a purin-free diet the chief factor determining the elimination of kreatinin is the weight of the person.

Sulphates.—The inorganic sulphates are slightly increased, varying from 3.6500 gms. to 2.9710 gms. The ratio of the total nitrogen to the sulphates is quite constant, being in the order of the observations as 1 to 4.7, 1 to 4.0, 1 to 4.1, 1 to 4.2, and 1 to 4.5.



The ethereal sulphates are not increased to any extent, as

compared with those values given by Folin in a normal person on the same diet, varying from 0.1800 to .29838 gms., whereas the latter values do not exceed 20 gms. Their values in the urine are quite constant. The increase is much less marked than that of the organic phosphates, and they show no tendency to vary with the intensity of the organic acidity, as the latter do to a certain extent. A curve to show this relation is here given.

The ratio of the preformed to the aromatic sulphates is not increased above the normal, being in the order of the days of observation as 13.1 to 1, 12.6 to 1, 19.1 to 1, 16.6 to 1, and 11.5 to 1, respectively. The urines were carefully tested in every case for the presence of indol and other products of putrefaction, but with negative results. This was thought necessary, because as H. Baldwin points out, in certain cases of intestinal putrefaction one of the aromatic products may be found in excess while the ethereal sulphates may be normal in amount or vice versa. In this case there is, therefore, no sign of intestinal putrefaction which might be considered to influence the interpretation of the results.

After the study on the Folin diet was concluded the total nitrogen, ammonia, fixed alkalies, total acidity and organic acidity were followed for a period of three days. The patient was on a mixed diet, but was at the same time started on the system of Fletcherizing all the food taken, being required to chew everything to a liquid consistency and being allowed to eat only for 20 minutes. Consequently during this period she received less than the normal amount of food, and the total nitrogen values fell. She also lost some weight in this period.

The organic acidity rose from 268.4 to a value of 594.5 and at the same time the ammonia coefficient also rose from 3.7 per cent to 5.7 per cent. Here again it is suggested that there is developing another periodic increase in organic acids. The fact that the organic acids appear in the urine to be increased periodically, does not of course necessarily mean a similar periodic production in the body, but may be simply a result of improved facilities on the part of the body for their excretion.

It seems suggestive in this case that though the acidosis is mild in degree, and far less extensive than that which occurs in many cases of diabetic coma, and though there is no evidence obtained of a marked disturbance in the alkalies of the body, the long continuation of such a periodic acidosis might well do vital damage to the body. Especially might this be true in cases where the afflicted persons subsist on a relatively insufficient diet, as many no doubt do, particularly with respect to the carbohydrate content. Here the body might be brought into a state of lessened power of oxidation, and then consequently not be able to destroy the abnormal acids formed, necessitating a call on its reserve store of the alkali-maintaining elements of the body.

The question as to how much light the condition of acidosis throws on the etiological problem of arthritis cannot as yet be answered. However, this much may be said that there is some connection between the condition clinically known as arthritis deformans and the occurrence of increased organic acids in the urine of patients suffering from this malady, as is demonstrated by the uniform findings, by H. Baldwin, of an increased organic acidity in 21 cases of arthritis deformans, and this confirmatory case of ours.

Why this disease should show a predilection for the joints and peri-articular structures is not clear, but the physiological knowledge that at these places the circulation is most sluggish may be of some value in attempting to explain this phase of the subject. It might be possible that the acids circulating in the blood would cause a local effect at the joints, proportionately greater than to the rest of the organism.

The fact that in our case no micro-organisms were found in the joint, of course, does not exclude their having been present at one time, and that they may have been responsible either directly or by the action of their toxins for the pathological changes in this disease. Moreover the findings of a chronic focus of inflammation in the tonsils, and the low opsonic index to streptococcus are most suggestive. In this case of arthritis deformans there appears to be a periodic acidosis which may be only a manifestation of a perverted metabolism, or may be more directly considered in the pathological state.

CONCLUSIONS.

(1) The metabolic study of this case of arthritis deformans seems clearly to indicate that there is taking place in the organism an acidosis, due to organic acids, and mild in nature.

(2) The acidosis is combated chiefly by the increased ammonia production, and the high ammonia ratio gives an index of the morbid process taking place.

(3) The disturbance in the elements maintaining the alkalinity of the body, the fixed alkalies, calcium, magnesium, and phosphorus is very slight, another indication that the acidosis is mild.

(4) There is an increase in the organic phosphates coincident with the increase in organic acids, the percentage of organic phosphates to the total phosphates reaching as high as 14.1 per cent of the total phosphates.

(5) There is a retention of calcium, magnesium, and phosphates throughout the experiment, which may be partly due to a diet abnormally rich in these elements, and partly to the pathological conditions in the body in this disease.

(6) No evidences of intestinal putrefaction were found in any of the urines, nor was the ratio of the preformed to the aromatic sulphates increased above the normal.

(7) The Folin diet is shown to contain abundant protein, carbohydrates, and fats for the maintenance of the body functions, as the patient gained 2½ pounds on it.

ANALYTICAL METHODS USED.

Total nitrogen, Kjeldahl.
Ammonia, Folin.
Uric acid, Folin.
Kreatinin, Folin (colorimeter method).

Inorganic sulphates, Folin as SO_3.

Ethereal sulphates, Folin as SO_3.

Inorganic phosphates, Titration by uranium acetate.

Total phosphates, Titration with uranium acetate, after incineration with sodium carbonate.

Calcium, gravimetrically by Salkowski as CaO.

Magnesium, gravimetrically by Salkowski as MgO.

Chlorides in urine by titration according to Volhard.

Chlorides in fæces gravimetrically as Cl_2.

Total acidity, Folin.

Fixed alkalies, Method of Edie and Whitley (Biochemical Journal, 1906).

Organic acidity, Method of Edie and Whitley (Biochemical Journal, 1906).

ANALYTICAL RESULTS.

	Day No.....	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
Food.	Total N..	16.60	16.60	16.74	16.22	16.22			
	Total P_2O_5	5.544	5.544	5.650	5.608	5.608			
	Total Cl..	6.260	6.260	6.200	6.630	6.630			
	Total SO_3	3.700	3.700						
	CaO	5.0460	5.0460	4.8220	4.2480	4.2480			
	MgO.....	0.3875	0.3875	0.34675	0.36738	0.36738			
Urine.	Total amount	1770 ccs.	1556 ccs.	1425 ccs.	2315 ccs.	1380 ccs.			
	Total nitrogen	15.36	14.51	14.06	14.77	13.21	8.54	9.90	9.19
	NH_3 nitrogen	0.545	0.585	1.025	1.122	1.748	0.313	0.388	0.526
	Uric acid	0.444	0.286	0.205	0.522	0.212			
	Kreatinin	0.710	0.555	0.600	0.600	0.628			
	Total Cl..	3.130	3.070	2.145	4.370	3.930			
	Total P_2O_5	2.600	2.650	3.290	4.390	3.270			
	Inorg. P_2O_5	2.226	2.524	2.988	3.897	2.970			
	Organic P_2O_5	0.374	0.136	0.302	0.493	0.300			
	Inorg. SO_3	3.230	3.650	3.440	3.450	2.971			
	Ethereal SO_3	0.24898	0.29838	0.1800	0.2076	0.24889			
	MgO.....	0.15978	0.11390	0.10744	0.11110	0.11312			
	CaO	0.8850	0.72101	0.88907	0.82842	0.69444			
	Fixed alkalies after incineration in urine.	ccs. N/10 −615.0	ccs. N/10 −435.0	ccs. N/10 −340.1	ccs. N/10 −652.8	ccs. N/10 −538.2	ccs. N/10 −207.9	ccs. N/10 −180.4	ccs. N/10 −293.8
	Total acidity	ccs. N/10 +537.0	ccs. N/10 +497.9	ccs. N/10 +441.7	ccs. N/10 +601.9	ccs. N/10 +485.7	ccs. N/10 +252.0	ccs. N/10 +302.8	ccs. N/10 +259.2
	Organic acidity	311.0	503.1	904.7	818.5	974.0	268.4	594.5	591.6
Food.	Total amount	gms. 10.500	gms. 21.900	gms. 34.000	gms. 20.000				
	Total N..	0.511	0.979	1.293	0.566	0.663			
	CaO	1.05677	2.5865	2.27390	1.72700	2.11220			
	MgO.....	0.15672	0.27249	0.25548	0.17357	0.22910			
	Total P_2O_5	0.4448	0.6240	0.5060	0.36770	0.36970			
	Total Cl..	1.0197	2.122	2.301	1.955	2.748			

In conclusion, I wish to express my indebtedness to Dr. C. Voegtlin for the many valuable suggestions and help during the course of this investigation.

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INDEX TO VOLUMES 1-16 OF BULLETIN.

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A METHOD OF PREPARING THE ROMANOWSKY STAIN.

By NORMAN MACL. HARRIS, M. D.

(From the Hull Bacteriological Laboratory of the University of Chicago.)

It it is with hesitancy that I present this brief paper dealing with a topic already so well handled by Wright, Hastings, and MacNeal, especially since there is nothing new to report in regard to staining reactions. My excuse, however, is the fact that in the methods of Wright and Hastings there is a relatively large amount of useful material thrown away; and that the method of MacNeal, to those not masters of technique, imposes a burden on patience, skill, and time, whereas with this method, these drawbacks are to a great extent eliminated and the resulting product is a very serviceable one.

The technique was elaborated through a study of the discussion of the subject by Michaelis, and is as follows: Make up a saturated solution of Grübler's watery yellow eosin in methyl alcohol (Kahlbaum's No. 1) and preserve; then using either "Koch's" "medicinally pure," or "Ehrlich's rectified" methylene blue as made by Grübler, weigh out two grams, powder in a mortar and add nine grams of sodium bicarbonate (c. p.) and mix thoroughly in a dry state, scrape out and place in a beaker of 250 cc. capacity, slowly mix in 25-30 cc. distilled water; cover mouth of beaker with filter paper and heat in an Arnold steam sterilizer for one hour and a quarter. There now remains a blackish fragmented cake and some excess of bicarbonate of soda; remove this black material to a mortar, grind it up and slowly add distilled water, decanting off into a well-stoppered litre bottle, and again add water to 200-250 cc. Any undissolved material is now to be put into the bottle and to the whole is added 10 cc. of a 4 per cent solution of sodium hydrate and then thoroughly shaken. The contents now assume a deep maroon color.

The next step is to extract with chloroform (commercial). For this purpose it is best to have at hand an extracting funnel[1] of 500 cc. capacity, though one of 250 cc. will do, and a couple of 500 cc. beakers or other suitable vessels, and a 15 cm. porcelain evaporating dish. To the contents of the liter bottle add at once 150 cc. of chloroform and shake moderately for 2-3 minutes, pour into the separating funnel and draw off the now deeply-colored chloroform into the beaker. The color of the watery solution should then be a pale blue, showing that extraction is practically complete; if still deep blue, add 3 cc. of the sodium hydrate solution, shake and further extract with chloroform.

Not infrequently it will be found that a portion of the chloroform becomes emulsified and will not separate. It must then be poured back into the liter bottle with the watery solution, 2-3 cc. sodium hydrate added, together with 50 cc. of fresh chloroform, and again shaken. Some emulsification is always present and a loss of 10 per cent of the chloroform usually results; however, by the addition of the 50 cc. of fresh chloroform, any resultant emulsified chloroform will contain so little extractive as to constitute no real loss.

It is now well to run the whole of the separated chloroform solution through the funnel once more to be sure to remove any of the original watery solution or emulsified chloroform that might have inadvertently gotten in. Then pour into the porcelain dish about 50 cc. at a time and evaporate completely over a water bath. As evaporation is nearing completion, often the sticky mass bubbles too actively and may spatter; this may be avoided by letting the water simmer gently for a while. Ultimately, the mass becomes dry, hard, of a deep violet-blue and covered with a brilliant yellow luster. This mass is made up largely of methylene violet, variable amounts of methylene azure and other substances.

Now scrape off as much as possible with a knife and put this into a glass-stoppered bottle of about 200 cc. capacity; add gradually, while stirring with a glass rod, to what remains of the extract in the dish some 30 cc. methyl alcohol (Kahlbaum's No. 1), and pour off into the bottle, filling the same three-quarters full with methyl alcohol. This constitutes the saturated stock solution of crude methylene violet and azure.

To make the staining fluid, take of the stock solution 66 cc., of methyl alcohol 33 cc., of the saturated methyl alcohol solution of "yellowy watery" eosin 1-1.5 cc. Bottle it and add from 0.05-0.15 gram of methylene blue.

The staining is carried out as in Wright's method, the coverslip or slide being not too scantily covered with the dye, which acts for one minute alone; then to this is added drop by drop a quantity of water equal to the bulk of the dye, after which the slide is allowed to stand for five minutes. Wash for 1-2 minutes in running water. This method stains excellently cells, and also brilliantly both the chromatin and protoplasm of trypanosomes (Tr. lewisi), Entamoebae histolyticae, and malarial parasites. If dysenteric stools are to be stained, the dye should be left in contact two minutes before mixing with water. To stain Treponema pallidium (Spiroch. pallida), carry out the staining as for blood films, but extend the time to ten minutes; the treponemata are readily found and well-stained of a pink-violet color. There is little or no precipitation if one is careful not to add too much water.

I have used the stain for a year past and have found it satisfactory. My hearty thanks are extended to Doctors Boggs, Cohoe, and Geraghty, of the staff of the Johns Hopkins Hospital, for the supply from time to time of blood films and other materials for study, and to Drs. J. H. Wright, T. W. Hastings, and W. J. MacNeal for samples of their stains used as comparative standards.

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[1] In the absence of a separating funnel the litre bottle may be converted into one by fitting to it a rubber cork through which are passed two glass tubes, one reaching to the bottom of the bottle, the other flush with the cork. By fitting to the former tube rubber-tubing and a pinch-cock, the chloroform solution may be completely drawn off on inverting the bottle.

THE OPERATIVE TREATMENT OF SUBDELTOID BURSITIS.

By W. S. Baer, M. D.

The part which injuries and inflammation of the subdeltoid bursa play in the restriction of motions at the shoulder joint has been brought strongly to our attention within the past few years. Formerly it was supposed that most if not all of the causes of partial or complete anklylosis of the shoulder were to be found within the joint. Roustan[1] in 1880 in a pathological study of periarthritis and bursitis of the shoulder divided his cases into acute, subacute, chronic, serous, purulent and fibrous bursitis. Jarjarvay,[2] in examining the cadavers of laboring men, called attention to the distinct thickening of the subdeltoid bursa and pointed out the traumatic factor in their etiology. Finney,[3] in the early days of this hospital, gave us a clinical picture of the affection and demonstrated a painful spot just below the coracoid process. It has remained for Codman,[4] however, in a very graphic paper to give us a complete anatomical demonstration as to the manner in which the inflamed subdeltoid bursa prohibits the full motion of the head of the humerus in the glenoid cavity.

Anatomy.—The subdeltoid bursa is about the size of a silver half-dollar and is composed of either one or two parts. When divided in two, the individual parts are sometimes spoken of respectively as the subacromial bursa and the subdeltoid bursa. The subdeltoid bursa, as its name indicates, occupies a very exposed position directly beneath the deltoid muscle. Its inferior surface is closely adherent to the joint capsule. It thus separates the deltoid muscle from the humerus and from the tendons of the long head of the biceps and the supraspinatus muscle which lie beneath the capsule. The walls of the bursa are very thin and it has the usual serous lining. As the arm hangs naturally at the side of the chest a small portion of the bursa lies beneath the acromion process, and this portion is spanned by the coracoid-acromial ligament. As one abducts the arm the bursa passes beneath this ligament. If you recall the relation of the coracoid and the acromion processes you will remember that the former lies on a much lower plane than the latter. So it is that the coracoid-acromial ligament prevents the swollen bursa from passing beneath it, and thus prevents the abduction of the arm to any degree. Together with Dr. Fayerweather I have injected the subdeltoid bursa with wax, the cadaver having been first immersed in hot solutions to allow freedom of motion. The wax in the bursa is then allowed to harden. Various motions of the shoulder joint were then attempted. It will be seen, as in the accompanying drawing, that if the bursa is slightly swollen all abduction, except the first ten degrees, will be prevented by the coracoid-acromial ligament. This ligament acts as an absolute hindrance to the further progress of the bursa beneath it. Now as we must have more abduction than this before we can internally rotate the arm to a sufficient degree to carry the hand behind the body it is easily seen why this is one of the characteristic restrictions in cases suffering from subdeltoid bursitis.

Etiology.—The causes of this affection are varied. When we recognize that the subdeltoid bursa is lined with a serous coat we must further recognize that it may be subject to all changes which are usually found in serous sacs, namely, serous effusions, inflammatory conditions from trauma and strain, infectious processes due to the gonococcus, tubercle bacillus, rheumatoid arthritis, etc. The main etiological factor, as shown by our cases, is trauma—trauma due to direct violence, trauma due to occupations which put the bursa under constant strain.

Pathology.—There are different pathological changes according as there are different etiological factors. In mild cases the walls of the bursa are perfectly normal, but there is an extra amount of fluid within the sac. In others the walls are slightly thickened. The lining membrane is hyperæmic and swollen, and the surfaces of the bursa are partially adherent by bands of fibrin. Some show complete obliteration of the bursal cavity, the walls being densely adherent, and at times more than 1 cm. in thickness, due to the new formation of scar tissue. If the process is a tuberculous one the cavity may be filled with a dense cheesy material.

Pain.—In the acute cases there is intense pain, but after the first few days of the disease pain is only present when an attempt is made to abduct the arm. At times the pain is worse at night owing to the inability of the patient to move the arm into any comfortable position. On pressure pain is generally elicited directly at the site of the bursa, but often it is referred along the bicipital groove or along the anterior aspect of the arm.

Swelling.—At times and especially in acute cases and in chronic cases where there is a marked thickening of the bursa a distinct swelling may be made out on the anterior aspect of the humerus just below the coracoid-acromial ligament.

Atrophy of muscles.—In long persistent cases there is an appreciable atrophy of the supraspinatus and infraspinatus muscles, and also at times of the biceps. These muscles, however, show a normal reaction to faradic and galvanic stimulation.

Restriction of motion.—This is the main physical sign of the disease and the one for which the patient seeks relief. In the older cases one can abduct the arm 10 to 15 degrees, but can go no further. The anterior motion of the arm is free, and the posterior motion is generally fair, but internal rotation is completely lost so that the patient cannot place the hand behind the body. Occasionally when the bursa is but moderately thickened, if one rotates the humerus outward so that the bursa is as far removed as possible from the coracoid

[1] Roustan: Montpellier méd., 1880, pp. 44 and 45.

[2] Jarjarvay: quoted by Putnam, Boston Med. and Surg. Jour., November 30, 1882.

[3] Finney: Johns Hopkins Hospital Bulletin, 1894, p. 83.

[4] Codman: Boston Med. and Surg. Jour., Vol. CLIV, p. 613, 1906.

end of the coracoid-acromial ligament, the arm can then be abducted to its full extent. As the bursa passes beneath this ligament one can feel a decided click just as one feels on the reduction of the congenital dislocation of the hip when the head of the femur goes over the posterior rim of the acetabular cavity.

Radiograph.—As a rule the radiographs in these cases are negative, and in this they allow us to make a differential diagnosis from cases of fracture of the greater tuberosity of the humerus which clinically are rather hard to differentiate. Occasionally, however, when the bursa is replaced by dense scar tissue there is a shadow to be seen in the radiographs at the point of the subdeltoid bursa.

Treatment.—What then shall be the mode of treatment of these cases? Let me here give an account of four cases which we have recently operated upon before discussing the question further.

CASE I.—G. M., male; age, 38 years; occupation, label cutter in a lithographic firm; admission, October 18, 1905. Complains of a painful shoulder and loss of motion in it.

Family history.—Negative.

Previous history.—Negative; denies lues and "tripper."

Present illness.—The patient's occupation is such that his right arm is constantly in action as he uses it with his machine in cutting labels. Eighteen months ago he first noticed pain in his shoulder. At no time has there been any redness or local heat, no evidence of any acute inflammation. The inability to use his arm has grown so bad that three months ago he was compelled to stop his work. His sleep is very much disturbed owing to his inability to get into a comfortable position.

Physical examination.—Very well-nourished man. Right arm hangs at his side. There is no particular atrophy of the supraspinatus or infraspinatus muscles, but there is a slight atrophy of the biceps. There is a slight fullness just below and to the outer side of the coracoid process. At this point it is somewhat tender and there is pain referred along the bicipital groove. As long as the arm hangs at the side there is no pain either on the internal or external rotation. The arm can only be abducted to 15 degrees and the patient is absolutely unable to put the arm behind his back. The radiograph shows the joint to be normal, but there is a slight blur near the greater tuberosity of the humerus, the site of the subdeltoid bursa.

October, 1905, under an anæsthetic I made an incision 4 cm. long midway between the coracoid and acromial processes running longitudinally to the humerus. The fibers of the deltoid muscle were separated and the subdeltoid bursa exposed. It was found to be somewhat thickened, the walls were injected and there were marked fibrous adhesions between the surfaces. The bursa was entirely excised. Cultures were taken and proved to be negative. The examination of the pathological specimen showed newly-formed fibrous tissue containing a great number of small blood vessels. In order to be sure that all the disability was due to the bursa an incision was made into the capsule and the joint explored. It was found to be entirely normal. The capsule was sewn with fine black silk. The muscle fibers were brought together with silk and the skin with subcutaneous silver wire. The arm was then put up in a splint in the position of abduction to 120 degrees and kept there for a week. This position was extremely trying. At the end of the week the splint was removed and the patient was allowed to go without any other support. Fourteen days after the operation he had a voluntary motion of 120 degrees of abduction, perfect inward and outward rotation, and could put his hand behind his back with perfect ease. He returned to his work on his fourteenth day. He reports to-night, twenty-one months after the operation. He has worked continuously at his trade ever since that time. The motion at the shoulder joint is perfect in all directions.

CASE II.—H. L., white; age, 23 years; admitted, January 21, 1907; occupation, plasterer. Complains of lameness in his left shoulder.

Family history.—One maternal aunt died of pulmonary tuberculosis.

Previous history.—Negative; denies venereal infection.

Present illness.—Six months ago while at work he noticed a pain in the left shoulder, which gradually increased to such an extent that he was obliged to stop his work, and he has been unable to do anything for the past five months. He cannot raise his arm without intense pain, which is referred along the anterior surface of the arm. Whenever he attempts to move the arm at night it is so painful that it immediately awakens him. He thinks the soreness is greater in damp weather.

Physical examination.—If the left arm is abducted 10 degrees a decided click is felt and the arm refuses to go any further in that direction. There is considerable amount of pain referred in the region of the bursa and down the front of the humerus. There is a decided fullness over the bursal region. There is practically no atrophy of any muscle.

Operation.—Under anæsthesia on January 25, 1907, I made an incision through the deltoid muscle as in the last case. The bursa was found to be much swollen almost 1 cm. in thickness. The walls were closely adherent and it was apparently one mass of scar tissue. Before removing the bursa the arm was abducted and the bursal sac impinged on the coracoid-acromial ligament and further abduction was prevented. The bursa was removed in its entirety and then full abduction was allowed. The muscle was closed with interrupted silk and the skin with subcutaneous silver. Owing to the discomfort caused by a splint, which would keep the arm at right angles as in the previous case, the arm was allowed to hang at the side and put in a Velpeau bandage. February 3, nine days after the operation the subcutaneous stitch was removed and the patient could bring his arm to a right angle. One week later, sixteen days after the operation, the patient could voluntarily bring his arm to the full amount of abduction, and had perfect use of it in rotation inward and outward. He went to work on his seventeenth day, having previously been out of work for five months. To-night, two and a half months after his operation, you see the motion is absolutely normal and has been so since his eighteenth or twentieth day. A culture taken from the bursa at the time of the operation proved sterile. The pathological report shows a mass of hard fibrous inflammatory tissue with much round cell infiltration.

CASE III.—A. G., white; age, 39 years; admission, April 22, 1906. Complains of inability to use her left arm.

Family history.—Negative.

Previous history.—Negative.

Present illness.—Fell on the ice and struck her shoulder three months ago. Since that time she has been unable to raise her arm at all and it has been impossible for her to get her hand behind her.

Complains of considerable pain along the bicipital region on moving the arm to the slightest degree. Her sleep has been greatly interfered with owing to inability to assume a comfortable position.

Physical examination.—Shows atrophy of the supraspinatus and infraspinatus muscles. She cannot raise her arm (that is abduct it) more than 10 degrees, and even this is very painful. Rotation inward and outward is materially restricted, though not to the same extent as abduction. Practically no swelling of the shoulder could be made out. The pain is referred down the front of the

arm. The radiograph is absolutely negative. May 1, 1907, the patient was anæsthetized and operated upon as in the first case. The bursa was found to be very thick, approximately 1 cm., and the walls densely adherent at the lower end of the bursa. At the upper portion of the bursa two walls were distinct, but they were much reddened and hypertrophied. The bursa was removed, and the closures were the same as before. The arm was put up in a Velpeau bandage. Two weeks after the operation the voluntary power of abduction was 90 degrees, and the rotation outward and inward was normal. At the end of the three weeks all motions were normal.

Case IV.—A. K., colored; age 46 years; admitted February 25, 1907; occupation, laborer. Complains of inability to use his right arm.

Family history.—Negative.

Previous history.—Negative.

Present illness.—Two months ago he fell twenty feet from a scaffold and struck on his right shoulder. The shoulder became swollen, but it did not keep him from his work. Three days later the swelling and pain had somewhat subsided, but he was unable to work owing to the inability to use his arm.

Physical examination.—Showed a slight swelling over the region of the subdeltoid bursa. There was no atrophy of the muscles. The pain was referred down the front of the arm and beneath the acromial process. The arm could be abducted to 15 degrees but could not be placed behind him.

Operation.—The patient entered the service of Dr. Halsted and I operated, as in the preceding cases, on February 26, 1907. The bursal walls were thickened and adherent one to the other so that the cavity was entirely obliterated. There had been a slight comminuted fracture of the greater tuberosity of the humerus, and the capsule and bursa were both adherent at that point. The bursa was removed and the capsule freed from the tuberosity. The wound was sewn as in the previous case and the arm put up in the Velpeau bandage. All dressings were removed on the eleventh day. On the fourteenth day the motion was greatly improved, abduction being allowed to 40 degrees, and internal rotation was almost perfect. To-day, seven weeks after the operation, he can abduct his arm to the right angle without pain notwithstanding the fracture of the tuberosity and the tacking down of the capsule.

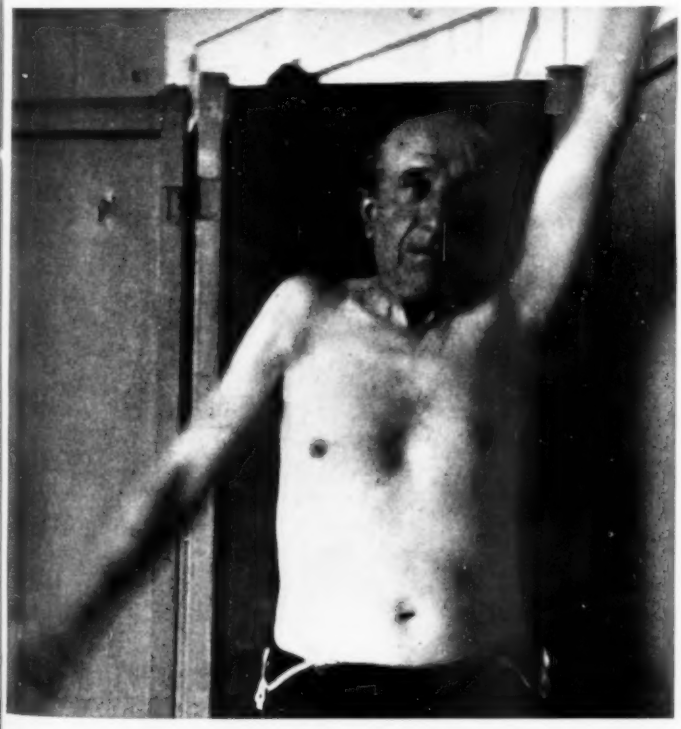
In the acute cases the treatment is obvious. Reduce the swelling of the bursa and relieve the pain. This may be done by the application of ice bags to the shoulder, or by the use of counter-irritants as the Paquelin cautery or hot air. If the inflammation is of rheumatic origin, the salicylates or aspirin prove valuable. Besides medication the arm should be put at rest to prevent traumatism of the bursa.

It is the more chronic class of cases which claims our especial attention in this paper—those cases which have resisted the treatment as indicated above, and have persisted for some time. I am sure we are all familiar with the clinical picture as shown in the cases just reported, and of the long and tediously painful course which they run, a course which often wears out both patient and doctor before they are relieved. Putnam[5] in 1882 recommended that the adhesions about the shoulder joint should be broken up by repeated manipulations under anæsthesia, and that passive motions and massage be given continuously. This we have found to be very trying to the patient, and indeed it takes a patient with great stability to submit to repeated anæsthetizations and continuous passive motions. In fact, many a patient has been brought to the verge of a nervous breakdown, if not actually pushed over the brink, by the constant pain which persistent passive motions necessitate. Codman's method of treatment is to break up the adhesions by forcible manipulations under anæsthesia, and to put the arm up in a splint which retains it in abduction of at least a right angle, and at the same time in rotation outward. The splint is left on for 24 hours continuously, and then for a week or more, daily taking the arm down for a brief respite. The idea is that this position of abduction and rotation will bring the raw surfaces opposite healthy ones. I think this method of treatment has its drawbacks. In the first place serious damage has been done by an attempt to break down the adhesions in this manner. Dislocations of the head of the humerus have occurred, as well as thrombosis of the axillary vessels. In the second place the position of marked abduction and rotation outward is tiresome in the extreme, if persisted in for any length of time. Further, adhesions will very often reform necessitating further manipulations and certain continuous passive motions for quite a period of time. Finally, there are certain cases where the bursa has been transformed into fibrous tissue of such a thickness that it would be absolutely impossible to pass it under the coracoid-acromial ligament. The method which I have outlined in this paper is the complete excision of the bursa. I do not believe with Codman "this bursa is indispensable in abduction and rotation of the humerus."

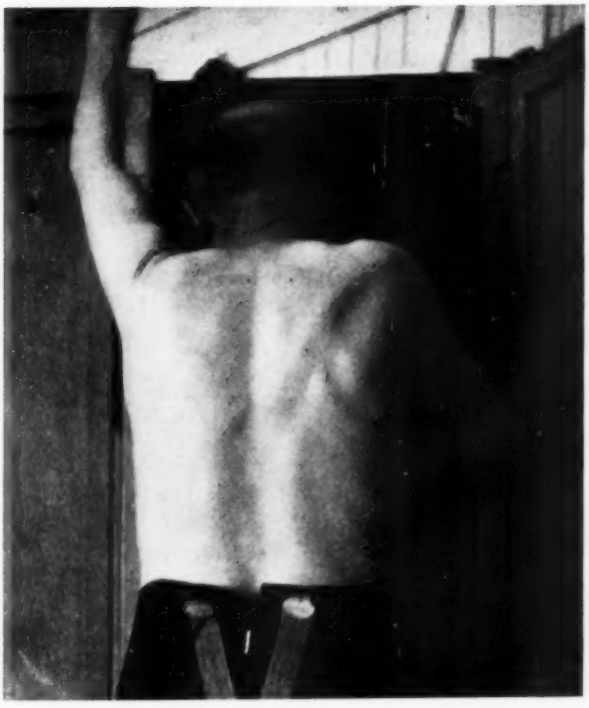
An incision of small extent should be made parallel to the long axis of the humerus midway between the coracoid and acromial processes. The fibers of the deltoid should be separated, and we come down on the subdeltoid bursa. This should be dissected out and removed in its entirety. If the walls of the bursa are at all thickened this is easily done, but if the walls are normal the sac is so thin that one is liable to overlook it. After the sac has been excised the arm should be put through all its motions, which one can then do, without fear of untoward results. The biceps muscle is brought together with fine black silk and the skin is sewn with subcutaneous silver. The arm should then be put up in a Velpeau bandage and dressed on the seventh day. The arm is then entirely freed and the patient allowed to use it at will. In reviewing our cases the advantages which the operative treatment affords are as follows: There is no fear of damage from tearing the adhesions, or rupturing the vessels, for all adhesions are removed in situ before the manipulations are commenced. The bursal walls cannot re-adhere, for they have been removed. The position at which the arm is placed at the side of the patient is most comfortable. The patient is saved a long course of passive motions and massage, which is very trying, and can return to work with full motion and painless use of the arm in from two to three weeks.

[5] Putnam: Boston Med. and Surg. Jour., November, 1882, p. 536.

CASE I.—Possible abduction 14 days after operation.

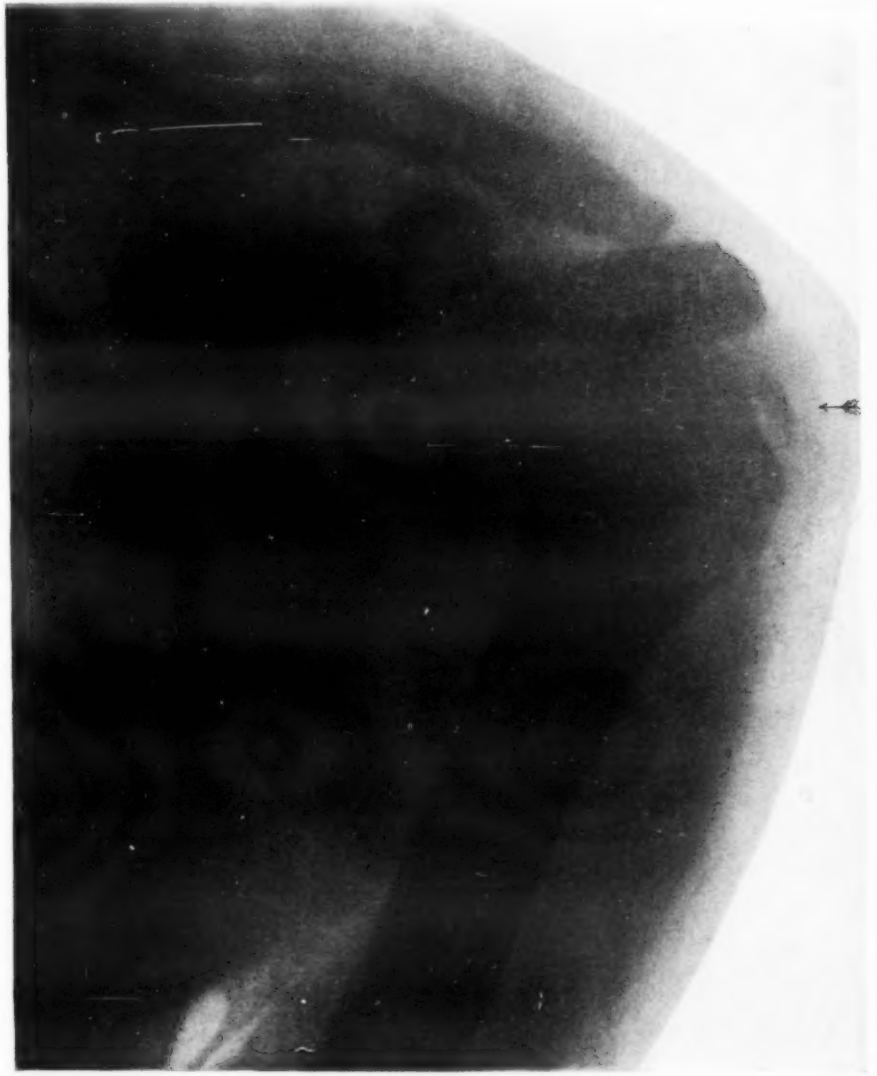


Both arms are abducted to their full extent.



Both arms are abducted to their full extent.

The arrow indicates position of bursa.



Fracture of greater tuberosity of humerus.

NOTES ON NEW BOOKS.

Modern Medicine. A System edited by WILLIAM OSLER, M. D., assisted by THOMAS MCCRAE, M. D. (*Philadelphia and New York: Lea Brothers & Co., 1907.*)

It is about twenty years since the appearance of the System of Medicine edited by the late Dr. Pepper. Systems are like pandemics, they appear only at long intervals. Some would say that they might be dispensed with; that we could get along very well with the endemic text-book or monograph. Of the great value of monographs there is no question but the profession as a whole pays little attention to them. The aim in a System of Medicine should be to have a series of articles which are to some extent on the order of monographs so that the practitioner can refer to a scholarly statement of the subject without the introduction of too much controversial or doubtful matter. Some men say that an article in a System is buried. As a rule it is, if it deserves burial. But good articles are constantly referred to and serve as a standard source of reference. Take for instance the article by W. H. Welch on Ulcer and Cancer of the Stomach in Pepper's System. No writer on these subjects since then can afford to neglect them. We know how often the teacher refers his students to various articles in Allbutt's System.

In this System Dr. Osler has drawn largely upon the young men in the profession; he has chosen his contributors rather from those who are breasting the hill than from those who have reached the summit or are looking toward the sunset. The general order followed in the volumes rather suggests that of some of the more recent French Systems. In arrangement and form it must be said that thus far the French have surpassed Germans, English, and Americans in the preparation of Systems of Medicine.

This volume opens with a short article on "The Evolution of Internal Medicine" by Dr. Osler. In this brief survey he notes the general forces which have influenced the development of our art from early times to the present. With this the aims of the present work are described and the hopes for the future. In the majority of his articles some references are given which demand search from the average reader. We wonder to how many the word *Nehushtan*[1] conveys any meaning.

Dr. Adami of Montreal writes on "Inheritance and Disease." As might be expected, he deals with a difficult subject in a clear and scholarly manner. Inaccurate ideas as to heredity and inheritance are common, which such an article should help to set right. Dr. Adami devotes special attention to a physico-chemical theory of inheritance under which emphasis is placed on conditions which affect the tissues of the parent and also the germ-cells.

The diseases caused by physical agents, light, heat, electricity, etc., are dealt with by Dr. Gordon. The tremendous increase in the use of electricity and the amount of work done with the X-rays renders these sections important. Under the subject of light and heat, writers do not seem to have arrived at a very clear separation of the various forms of disease. Dr. Edsall has given us in the section on diseases caused by chemical agents a most satisfactory discussion of the subject. He draws attention to the fact that in this country there has been no official effort directed toward the study or control of dangerous trades. Throughout his articles there are references to the regulations of other countries which suggest the importance of similar legislation here. The diseases caused by alcohol, opium, and cocaine are discussed by Dr. Alex. Lambert of New York. His work in the Bellevue Hospital has given him a wide experience and the lesions should be well-marked when we read that "not infrequently, at least among the Bellevue Hospital patients, a pint (of cheap whisky) is reckoned as a single drink." He discusses the various doubtful points as to the effect of alcohol in a fair spirit and without the bias evident in so many articles. Special attention is drawn to the symptom-complex of "wet brain." The notice given to opium smoking suggests a greater prevalence in one or two large cities than in the country generally.

Dr. Novy discusses Food Poisons and it is interesting to note how little relative importance is given to ptomaines compared with bacterial infections. The greater part of the article deals with bacterial poisons. Dr. Noguchi takes the subject of Snake Venoms on which he has done so much work.

The subject of Auto-intoxications is taken by Dr. A. E. Taylor. It is not possible to review this article in detail, but it is to be hoped that it may help to clear away many of the erroneous views regarding this subject. Many of the profession have accepted statements for which there was no basis and have made the term "auto-intoxication" a convenient diagnosis for obscure conditions and sometimes for conditions not so obscure. The gastro-intestinal auto-intoxications are taken up first and it is interesting to see how little accurate basis there is for the many views held regarding their importance. Abnormalities in oxidation, retention intoxications, and those in relation to salts, acids, and alkalies are discussed. In the second chapter on this subject the auto-intoxications associated with the metabolism of the various groups of foods are gone over. In some senses, gout and diabetes may be regarded as coming under the heading of auto-intoxications. Altogether, Dr. Taylor has written a most satisfactory article on a difficult subject.

Dr. J. H. Wright contributes the section on Actinomycosis and Nocardiosis, which he is well fitted to handle from his thorough work on them. Introductory to the Diseases caused by Protozoa are sections on the Protozoa by G. W. Calkins and on mosquitoes by L. O. Howard; each is an authority on his subject. The article on Malarial Fevers is by Surgeon C. F. Craig of the U. S. Army. In the discussion of the æstivo-autumnal parasite he takes the view that there are two varieties, the quotidian and tertian. This follows the opinion of many Italian observers, which, however, is not held by many in this country. That subject of much discussion, Black-water Fever, is discussed by J. W. W. Stephens of the Liverpool School of Tropical Medicine. He argues strongly for the influence of malarial infection and considers Black-water Fever not as a disease *per se*, but a condition of the blood *produced only by malaria* in which quinine, other drugs, cold, etc., may produce a sudden destruction of red cells. Col. David Bruce of the British Army writes on Trypanosomiasis, on which he has done so much work. Dr. R. P. Strong of Manila has the section Amœbic Dysentery and contributes an article drawn from experience both in this country and the Philippines. He directs attention to the association of intestinal hæmorrhage and liver abscess in amœbic dystentery.

The section on Animal Parasites is written by Dr. C. W. Stiles, who has made a splendid article without too much detail and yet very complete. Until now we have lacked such a comprehensive discussion which was readily available and this impresses us as the best article in English on this subject and should be of great value to the general practitioner who has not access to the more elaborate works.

The section on metabolism opens with an elaborate discussion on Nutrition by Professors Chittenden and Mendel of Yale. The work of the Yale School on this subject is well known and they have given a comprehensive article in which the general considerations are first discussed and then the various modifications of metabolism under changing conditions. The changes which the various foods undergo and especially the importance of inter-

[1] To save the reader's time, he is referred to 2 Kings xviii, 4.

mediary metabolism are fully taken up. With this the question of the disorders of metabolism is closely associated. As is well known, Professor Chittenden holds strongly that for many persons "the nutritive demands are certainly more than satisfied by the customary dietaries." Whether one is willing to go the length to which he does, there can be no question of the importance of the matter and the necessity of careful study of this subject. The article closes with the discussion of the composition of the various foods in their relation to nutrition and the constituents of various systems of diet.

The articles on Diabetes Mellitus and Insipidus, and Gout are by Dr. T. B. Futcher. These are most thorough and satisfactory, and may be pointed to as examples of what System articles should be. Dealing with diseases characterized by complicated chemical disturbances, the information on these is set forth with such clearness and detail that no one can fail to understand. The clinical side is well gone over and fully considered. The discussion of the treatment of these diseases is very rational, that on Diabetes Mellitus especially so. In Diabetes Insipidus it is interesting to note the great importance placed on syphilis as an etiological factor. The articles by Taylor, Chittenden and Mendel, and Futcher all deal to some extent with the questions of carbohydrate and protein metabolism. It is interesting to compare these and see the points of view which result from the different aspects which each considers.

Dr. J. M. Anders deals with obesity and gives full discussion to the modes of treatment. Dr. Still of London writes on Rickets both in early life and in its late manifestations. Dr. Hutchinson of London contributes the section on Scurvy. Both of these are comprehensive articles.

Altogether this volume is most satisfactory and sets a high level for the succeeding ones to maintain. There is so much which might be commented on that it is difficult to choose. Dr. Osler has been fortunate in many instances in having contributors who have specially identified themselves with the subjects which they discuss. As he says in his Introduction, it is not always easy for men who are saturated with their subject to keep within limits and remember the practical character of the men who read, yet we think that the contributors have kept this in mind and succeeded.

The publishers have done their work well. The page is attractive, the type is clear, the illustrations are well done, and the book lies open well at any page, a quality as admirable as rare.

Diseases of the Lungs. By Robert H. Babcock, A. M., M. D. First Edition. (*New York: D. Appleton & Co., 1907.*)

This is by the author of the splendid work on the "Diseases of the Heart and the Arterial System" (Appleton, 1903) and is intended to complete the work on the "Chest." As the author explains in his preface, the object of the book is to present "these as well as other subjects in a manner that will render them available to the student and practitioner of medicine, since the book is not intended for the experienced internist, whose knowledge may surpass that of the writer. There is nothing novel in method, and no wearisome discussion of unfounded theories. The aim has been to be practical and hence ætiology, diagnosis, and treatment have received especially full consideration."

We may say that the author fully lives up to his words throughout the whole book, which is to be specially commended. The work is of about 800 pages, being divided into three parts, the first is devoted to the "Bronchi," each chapter covering its separate field most thoroughly and especially in regard to treatment. The author uses the term "Plastic Bronchitis" in place of the older one "Fibrinous" on the assumption that we are not certain in all cases that the casts are composed of fibrin.

The second section is divided into the diseases of the "Lungs" proper and covers about 550 pages. The subject of Pneumonia (lobar) is considered under the name of Acute Fibrinous Pneumonia, which the author chooses instead of Lobar, as being more correct and at present in wide use among the Germans. Fibrinous Pneumonia was the term chosen by Virchow, on account of the richness of the exudate in fibrin. In speaking of the designation "lobar" the author says: "It is now known that the local process may have a lobular as well as a lobar distribution, and, therefore, this term is not scientifically accurate." It has the sanction of usage, however, because agreeing with the facts in most cases, and is the name most commonly given to the diseases in this country. As stated, he says he will use the terms lobar, croupous, and fibrinous interchangeably in the article.

The whole article on Pneumonia is splendidly written, being extremely clearcut and logical; the chapter on treatment is especially timely and the danger of overdrugging is forcibly brought out, and fresh air is given its most important place in the modern management of patients. The author cites many cases from his hospital and private practice, which illustrate many points and are interesting, but in some instances a little too lengthy.

The section on the lung disorders is thoroughly up to date and complete, that on Pulmonary Tuberculosis in particular; in the ætiology of this disease, the author has an especially valuable chapter and touches well on the conditions of life and environment. The story of the poor, and of tenement life in the great cities is appealingly and forcibly told. The symptomatology and diagnosis are well brought out, the prevention and treatment exhaustively considered, and the educational side of the question, with the relation of the state and municipal control is well accentuated. The sanatorium, dietetic, and climatic treatment is most completely detailed, and "drug therapy" considered, as the author says, "not for the purpose of its indorsement, but to condemn it in the most emphatic terms possible."

In closing the section on treatment the author gives good brief summaries of Tuberculin and its actions, the different serums, and the production of artificial immunity.

The general diseases of the lungs are all considered at some length and most thoroughly, and in the last section the inflammations and diseases of the Pleura are treated of in about 80 pages most carefully.

In the first edition of any work numerous typographical errors are bound to creep in; among these we may mention the following:

In the table of contents, p. x, the line under treatment of pneumonia should read *with a physiological salt solution.*

On p. xii heading of chapter xxviii, should read *Pneumonic.*

In the text, p. 210, bottom line, the word *râles* is left out.

On p. 618 the 12th line from bottom *antitoxic* should be put in place of toxic.

On p. 77 fig. 8 has no reference to it in the text.

On p. 443, fig. 54, *upper lobe* should read *lower lobe.*

As regards the illustrations they are as a rule well reproduced and represent clearly what the author desires to show; the photographs are good, but the plates are in some instances a little too diagrammatic.

The book as a whole is very well gotten up by the publishers and can certainly be commended highly "to the practitioners and students of medicine," and will serve as a splendid companion volume to the author's work on the Heart and Arterial System.

J. A. Chatard.

Tuberculosis, as a Disease of the Masses and How to Combat It. By S. A. Knopf, M. D. Fourth Edition. (*New York: Published by Fred. P. Flori.*)

A brief but clear and satisfactory supplement on Home and

School Hygiene is the only important addition to the last edition of this "Prize Essay." "The short historical review of the anti-tuberculosis movement in the United States from its beginning to the present day," which has also been inserted by the author, seems to the reviewer out of place in this pamphlet. There is nothing new to be said about the original essay whose value is measured by its world-wide fame, and the call for a fresh appearance.

R. N.

A Treatise on Surgery. By GEORGE RYERSON FOWLER. Vol. I. *(Philadelphia: W. B. Saunders & Co., 1906.)*

The book, including the index, comprises 722 pages, with 398 text illustrations and four colored plates. The paper is good, the type large and clear, the illustrations well executed, and altogether the mechanical construction of the book is very creditable.

The author states in his preface that he will treat the subject of surgery under what he designates as the Anatomic Method, that is, the consideration of injuries and diseases as related to each particular region. This is the same method which was followed by Koenig.

The first chapter, on Inflammation, includes Surgical Bacteriology and a short discussion of Sterilization and Aseptic Technique. This chapter in many parts is incomplete and the illustrations comprise those of ordinary sterilizers, incubators, etc., which are well known to every second-year student.

The section on "Laboratory Aids" includes a synopsis of the examination of the blood, urine, feces, gastric contents, etc. Illustrating this chapter one finds the ordinary blood-estimating apparatus, the freezing microtome, and other things equally elementary. To any one who is familiar with clinical microscopy such a short consideration will be of no value, and to others who are not so well grounded his descriptions are too cursory to be of any material aid. In a discussion on leucocytosis it is his opinion that the increase of leucocytes in general indicates a resistance on the part of the patient rather than any special local condition. In order to arrive at an idea of what has taken place at the point of infection an estimation of the polynuclear neutrophiles must be made. From an examination of over 1400 blood counts he has found the normal to be 70 per cent. Above this number he interprets as indicating the presence of pus; below this number pus or gangrene are excluded. An increase of the polynuclear neutrophiles with little or no inflammatory leucocytosis indicates an inflammatory process, together with little resistance on the part of the patient. The same increase with a high inflammatory leucocytosis means good resistance. In this chapter he has given two cuts; one of tubercle bacilli and another of smegma bacilli. The tubercle bacilli are beautifully arranged in regular lines and curves; the smegma bacilli irregularly scattered in patches. Such a distinction, no doubt often occurs, but not sufficiently so to make this any special point in differentiating them.

Anæsthesia is well treated, but too little is said about Nitrous Oxide Gas and Ethyl Chloride.

In the section on General Principals of Operative Technique he has followed the same course of illustrating the simplest surgical instruments; knives, dissecting forceps, saws, curettes, etc. In modern surgical education a student becomes familiar with these instruments by seeing them and not by their illustrations in a book.

The discussion of Tumors is done in a very general manner and so extremely cursory that it has not much value.

The general consideration of Bone Diseases is incomplete.

Syphilis and Tuberculosis are treated in a short and elementary manner, for such important subjects.

The book presents very little that is new and is cumbered by so many unnecessary illustrations, incomplete chapters and descriptions of unnecessary detail that it is questionable if it is any addition to surgical literature.

Prevalent Diseases of the Eye. By SAMUEL THEOBALD, M. D. *(Philadelphia: W. B. Saunders & Co., 1906.)*

The teaching of the special branches of medicine has assumed a rank of growing importance at our medical schools. There are those still in practice who in their student days had the entire scope of theory and practice covered by the "five chairs." Later on the many "ologies" claimed recognition and so during the past half-century, the length of the course of study was doubled, but the separate branches taught increased fivefold. With this growth in the number of separate studies, there has been little effort at coordinating them. The teaching of the different branches, as reflected in the numberless text-books, has been apparently guided by the purpose of furnishing the student with a more or less condensed supply of the entire subject, varying between the limits of the inadequate compends and the ponderous hand-book. And yet it has been the object of the medical school to prepare the vast majority of its graduates for general medical practice. Considering the vastness of the field, it would appear quite as important that the teachers of the specialties should study carefully to exclude that which is unimportant, not in itself, but from the point of view of the needs of the general practitioner, as to teach that which is important. For it need scarcely be added, that while much of the knowledge of a specialist would be ornamental only to the general practitioner, there is that which is not only useful but essential. And just as the teacher must use the art of selection, so the text-books, which are designed for the student and the medical practitioner, should be adapted to their needs. Of no specialty can it be said more truly than of ophthalmology that "of making many books there is no end," and yet few measure up, even partially, to the requirements as just outlined.

In the recent book of Dr. Theobald, "Prevalent Diseases of the Eye, a reference hand-book, especially adapted to the needs of the general practitioner and the medical student," the attempt has been made to meet this demand of the general practitioner "by taking into account the fact that the great majority of physicians are not skilled in the use of the ophthalmoscope, and are not likely ever to be; that they possess neither the experience nor the necessary paraphernalia to make trustworthy tests of refractive errors, of muscular anomalies, or of the visual fields; and that they are not qualified to perform, and have no desire to undertake, the more delicate eye operations . . . . but, on the other hand, that there are other important affections of the eye which he is competent, or should be competent, to diagnosticate correctly, and to treat successfully."

The book is admirably written, with great clearness, reads smoothly, and is specially interesting because it records the wide experience of a careful and observant practitioner. It is refreshing to read a new text-book which is evidently not a simple compilation of those that have gone before. The illustrations are numerous and clear, the colored ones, mostly original, are especially vivid.

The chapters on general observations upon the diagnosis of diseases of the eye; description of the methods of examining the eye available to the general practitioner; general observations upon the treatment of diseases of the eye; and those devoted to the external diseases including diseases of the iris, are admirable. The chapter on anomalies of refraction and accommodation gives good advice concerning the prescribing of glasses, advice it would be well for many physicians to read.

In some matters the work does not follow the accepted views, the differences being based upon the experience of the author. He has succeeded in laying stress upon the subjects which are of importance and eliminates that which is unimportant. The book

is filled with helpful hints, for the author does not despise minutiæ, and it will certainly prove useful and become a popular work of reference to the physician in general practice.

H. F.

International Clinics. Vol. I. Series 17. 1907. (*Philadelphia and London: J. B. Lippincott Company, 1907.*)

In addition to a number of interesting papers on treatment, medicine, surgery, gynæcology, ophthalmology, and laryngology, more than a hundred pages of this volume are devoted to the progress of medicine during 1906. As a historical review of the advances made, and the constant change of opinion and practice in various branches of medicine, this final chapter is perhaps the most important in the book. The first article "On the Psychic Treatment of Some of the Functional Neuroses" by Dr. Lewellys F. Barker is, however, one of equal value from another point of view. These troubles are to-day being studied with more care than ever before, and are securing the proper attention which, through ignorance and misunderstanding of their importance, they long failed to obtain. To save a neurasthenic or hypochondriacal patient from becoming insane, or to rescue the so-called "border line" sufferers, and restore them to a normal frame of mind is of more value to the community than to cure a case of pneumonia or typhoid fever. It is, therefore, necessary that as much study be paid to the "functional neuroses" as is now spent on other more acute diseases. Every authoritative article, therefore, is to be welcomed, and it is satisfactory to note that the "International Clinics" begin their new series with a contribution to this subject.

R. N.

The Diseases of the Nose, Throat, and Ear. By Charles Prevost Grayson, A. M., M. D., Clinical Professor of Laryngology in the Medical Department of the University of Pennsylvania, etc. Second edition, revised and enlarged. (*Philadelphia and New York: Lea Bros. & Co., 1906.*)

We take pleasure in recommending the second edition of this well-known work to students and practitioners of medicine. It is hardly comprehensive enough to be classed with the larger works devoted to special practice, but is most useful as a reference book for those who have not the time to consult the more exhaustive works. The sections devoted to local treatment are of especial value.

We cannot agree with the author in some of his statements. He decries the use of cocaine solutions of greater strength than 8 per cent. We have found that one cannot get nearly as satisfactory anæsthesia with such solutions as with the powdered cocaine, especially in the case of operation on the sinuses.

We would like to call the author's attention to the incorrect use of the term myxoma in the chapter on growths in the nose. He uses this expression in describing the common mucous polyp found in the nose. Myxomata are the rarest of all tumors found in the nose, mucous polypi the most frequent. The fluid content of the mucous polyp is not mucin but a serous exudate and the technical name of the mucous polyp is edematous fibroma.

The chapters on ear diseases are fairly satisfactory. We regret that the author has not seen fit to describe in a separate chapter the fairly common disease known as otosclerosis. This differs in several important ways from the frequently seen chronic aural catarrh. The middle ear is not affected with the exception of the stapes, which is united by spongy bone to the oval window. This disease usually starts between the twentieth and thirtieth years of life with gradual loss of hearing and tinnitus. It should be sharply differentiated from the catarrhal condition as the prognosis in the latter is so much more favorable.

Sylvan Rosenheim

Department of Neurology: Harvard Medical School. Vol. II. Boston, Mass., U. S. A., 1907.

From this department there has just appeared a second series of contributions by such well-known neurologists as Putnam, Knapp, Walton, Taylor, and others, dealing with hysteria, cerebral and cerebellar tumors, injuries to the skull and cord, multiple sclerosis, exophthalmic goitre, tabes, and psychotherapeutics. The collection of papers published in various journals which all deal with one branch of medicine is to be commended, and these volumes from the Harvard Medical School, with a similar volume lately published on "Tumors of the Cerebrum," by Dr. Mills and others in Philadelphia make most interesting additions to American neurology.

R. N.

Epitome of Pathology. By John Stenhouse, M. D., and John Ferguson, M. D. (*Philadelphia and New York: Lea Bros. & Co., 1906.*)

This little book of 265 pages is intended to be an epitome of general and special pathology. The section on general pathology has a few fairly good illustrations. The subject matter is chiefly clinical chemistry and microscopy, and for the most part is little more than a dictionary. The second part on special pathology states briefly the etiological factors of and the anatomical findings in the various diseases. If the authors had been more careful with their English, the book would be more valuable, since a person knowing little of the subject would certainly be considerably misled in many instances. The authors have also made bad mistakes in stating the size of the various intestinal parasites (see page 92).

Talks to First-Year Nurses. By Alfred T. Hawes, M. D. (*Boston: Whitcomb & Barrows, 1907.*)

As the author says in his introduction "the following talks are elementary in character, and are not to be considered as thorough discussions of the subjects of which they treat. The details are to be filled in by subsequent studies and courses of lectures. The design of these talks is to give nurses enough information to enable them to understand the general condition of their patients and to carry out intelligently the details of their work."

Although many primers or hand-books for nurses have been published, yet there are but one or two really first-class ones, and this small book cannot be considered to rank with the best. It is too "elementary" and does not give "enough information." Too many subjects are cursorily handled and it is not altogether well arranged. In one respect it resembles too closely a dictionary in simply defining names and terms. Its few illustrations are poor. The author's style is clear and simple, but in attempting to be simple and brief he has cut his work too fine and so failed to produce a really serviceable guide.

R. N.

The Prophylaxis and Treatment of Internal Diseases. By F. Forchheimer, M. D., Professor of Theory and Practice of Medicine and Clinical Medicine, Medical College of Ohio. (*New York and London: D. Appleton & Co., 1906.*)

There is undoubtedly a demand for a good work on the prophylaxis and treatment of internal diseases. Many students and practitioners, particularly recent graduates, feel the need of more detailed information than that which can be given in the usual text-books of medicine.

In the present volume the subject-matter is treated in the usual sequence and the individual topics are, as a rule, discussed at length. As in most first editions, all is not as the author may have intended. The treatment of "typical" lobar pneumonia is well given. Separate consideration is given to "atypical" pneu-

monia; the author says: "All forms of lobar pneumonia belong to this type when their course varies from that of the normal as to the duration, intensity, complications, and the individual attacked"—clearly a too elastic definition, one which, we should judge, might be made to include the majority of all cases of lobar pneumonia. In the atypical pneumonia "quinine, given by the mouth or subcutaneously, is of decided value"—a statement to which many would take exception. Though the author has not used the drug himself, it is disappointing to find reference made to creosote carbonate which has been of no value in the hands of most careful observers. That "adults need never be immunized" against diphtheria is doubtless an erroneous view and one which, if followed, would lead to serious consequences. In the prophylaxis of rheumatic fever the value of removal of the tonsils has been overlooked, though this may have been purposely omitted because of the lack of satisfactory statistics upon which to base judgment. The classification of diseases of the stomach is confused, particularly that relating to the gastric neuroses. Under motor neuroses the only condition treated of is cardiospasm, while peristaltic unrest, nervous vomiting, regurgitation, etc., are placed under the secretory disturbances. The nervous form of achylia gastrica is generally recognized, but "cancer, tabes, and sclerosis of the stomach" are not so generally accepted as causes of it. The chapter on chronic myocardial insufficiency is one of the most illuminating in the book. Here the author is seen at his best. The subject is handled excellently, the various methods of physical therapy are splendidly discussed, and the advice given seems thoroughly sound in all respects. Criticism might be made of the use of the word "decompensation" for the usual "incompetency"—a minor point—but, aside from this, it is difficult to see how the text could be improved. Certainly great good would result if all practitioners could read and profit by what the author has to say on chronic myocardial insufficiency. Treatment of tetany with thyroid extract is irrational therapeutics, and from the excellent results reported in the few cases where parathyroid glands have been tried, they should be administered in this symptom-complex. Neurasthenia, one of the commonest—perhaps the commonest—of diseases, is unintelligently or unsympathetically treated by the majority of physicians, it is safe to say. While it must necessarily be considered within the limits of a few pages in a work of this size, the space has been very advantageously used and the teaching is, we believe, helpful.

As in all of Appleton's works, a good quality of paper is used, the type is easily legible and clear, and the book is attractively put together.

A Text-book on the Practice of Gynecology. By WILLIAM EASTERLY ASHTON, M. D., LL.D. Third edition, revised and enlarged. (*Philadelphia and London: W. B. Saunders & Co.*)

The third edition of this work on the practice of gynecology has been demanded inside of a year. This tribute to its worth speaks for itself. The present third edition has received a thorough revision by the author, and many beneficial changes have been made. The general plan of the book for teaching purposes is excellent. Dr. Ashton has followed a definite routine in his consideration of each subject, taking up the different phases of each disease in a definite order. Such an arrangement is always helpful to the student.

In a practice of gynecology such as this book is, one could hardly expect to find long chapters on the fundamentals, such as embryology and pathology. However, as both of these subjects, especially the latter, are at present so intimately connected with the actual practice and operative procedures in gynecology, it seems as though some mention should be made of the pathological changes. One might almost say that instead of the minute descriptions of operating rooms and operative technique, which can never be learned from a book but only by actual practice in the operating room, possibly that space might have been used in brief descriptions of the pathology of gynecological affections.

The chapter on cancer is very full, and strongly emphasizes the important points in the diagnosis of this disease. The early symptoms are plainly stated and their importance is insisted upon. Clear statements such as Dr. Ashton makes in this chapter on the recognition of cancer are the very words needed to train the general practitioner and the student to bring cases of cancer of the uterus early to operation.

Each disease of the pelvic structures has been treated separately and distinctly, and there has been no lumping of subjects with many subdivisions. This feature of the book is especially satisfactory and prevents confusion in looking up any one subject.

The chapter on cystitis is excellent, especially the differentiation of the various kinds of cystitis, separating them one from the other clearly.

In the chapter on tuberculosis of the pelvis, perhaps more stress might be laid on the radical operations for tuberculosis. Pathology shows us that tuberculosis of the tubes and ovaries rarely occurs without the involvement of the uterus, especially the endometrium. Dr. Ashton advises complete removal of the pelvic structures when there is present macroscopic evidence of involvement of the uterus. However, as the tubes and ovaries are so seldom, if ever, involved by tuberculosis with uterus remaining free, it seems that the greatest chance for a cure would be by the complete removal of the entire pelvic structures in *every* case of pelvic tuberculosis.

The chapter on the anatomy of the pelvic floor is especially important, and the illustrations, while somewhat diagrammatic, are clear and helpful.

The book is a satisfactory one from the standpoint of the student and the general practitioner, for whom it is written, and forms a good every-day reference book for workers in the gynecological field. H. T. H.

Anatomical Terminology with Special Reference to the BNA. By LEWELLYS F. BARKER, M. D. (*Philadelphia: P. Blakiston's Son & Co., 1907.*)

The need for a consistent, methodic, and exact system of terms used in designating anatomic parts and their position has been felt by many teachers and investigators. Recent advances in anatomy have been attended by many changes in nomenclature and anatomic names have accumulated in such large numbers that our lexicology has become tremendously overburdened. It is a strange trick of the vicissitude of things terminologic[1] that the reform movement initiated as it was on this side of the Atlantic in the early '80's under the leadership of Wilder, Leidy, and others should result in the production of a list of terms adopted by the "Anatomische Gesellschaft" of Germany in 1895 under the title of "BNA" and in this year of 1907 to be brought, more effectually, we trust, to the attention of American students under the editorship and with the stamp of approval of Professor Barker. In the preface of this book we find the history of the work done by the German Commission up to the time of the adoption of its list at the Bâsle meeting. Rather scant reference is made to the prior revisions undertaken by committees of the American Association for the Advancement of Science, the Association of American Anatomists, and the American Neurological Association. In this connection Professor Barker expresses the hope that "at another revision, the terms in Professor Wilder's lists which differ from those of the 'BNA' may be carefully considered, and that his terms, where they are better than those of

[1] *Terminology* is a hybrid Latin-Greek word (as are many of the "BNA" terms) and should be replaced by *nomenclature*. For other philologic reasons, *anatomic* is preferable to *anatomical*.

the present 'BNA,' may be adopted." While we disclaim all desire to revive the animadversions of a past controversy, it must be pointed out that this is written more than a decade after the American pioneers in the reformation of anatomic nomenclature met with rebuff, even discourtesy, at the hands of certain members of the very commission which formulated the "BNA."

In the pursuit of its work the Commission adopted certain general rules of simplification which are in the main excellent ones. Thus "(1) each part shall have only one name; (2) each term shall be in Latin and be philologically correct; (3) each term shall be as short and simple as possible; (4) the terms shall be mere memory signs and need lay no claim to description or speculative interpretation; (5) related terms shall, as far as possible, be similar—e. g., *Femur, Arteria femoralis, Vena femoralis, Nervus femoralis;* (6) adjectives, in general, shall be arranged as opposites—e. g., *dexter* and *sinister, major* and *minor*, etc."

A divergence of opinion among the members of the Commission with regard to the use of personal names was compromised by putting the widely used ones in brackets after the objective name—e. g., *Lig. inguinale* [*Pouparti*]. The Commission did not adhere strictly to this rule as shown by the terms *Corpus Wolffi, Ductus Wolffi*, and *Ductus Muëlleri;* unwarranted terms we think, in the case of the first two at least, since morphologists have long employed *Mesonephros* and *Mesonephric duct*. While in the "BNA" the older *Foramen of Winslow* is correctly replaced by *Foramen epiploicum* (ἐπίπλοων = omentum) we question the correctness of the term *Appendices epiploicæ* (p. 48), structures which have nothing to do with any omentum.

Although the Commission adopted the general rule that "each term shall be as short and simple as possible" we do not find this resolve carried out to the most desirable extent in many instances. The attending difficulties may be of natural origin since the German-speaking anatomist enjoys the facile enunciation of such terms as "Atrioventrikularverbindungssystem," "Brustschlüsselzitzenfortsatzmuskel," and "Zwischenkiemendeckelstück." That the "BNA" terms chiefly lack the attribute of brevity may be seen in their comparison with certain terms in Wilder's lists, tabulated here below, and more or less extensively used by leading American teachers and investigators:

"BNA."	*Wilder.*
Calvaria	Calva.
Chiasma opticum	Chiasma.
Nucleus dentatum	Dentatum.
Corpus pineale	Epiphysis.
Medulla spinalis	Myelon.
Medulla oblongata	Oblongata.
Corpus striatum	Striatum.
Corpus callosum	Callosum.
Calcar avis	Calcar.
Falx cerebri	Falx.
Falx cerebelli	Falcula.
Tentorium cerebelli	Tentorium.
Dura mater	Dura.
Pia mater	Pia.
Splenium corporis callosi	Splenium.
Vena cava superior	Præcava.
Vena cava inferior	Postcava.
Substantia corticalis	Cortex.
A. cerebri anterior	A. præcerebralis.
Nucleus amygdalæ	Amygdala.
M. latissimus dorsi	M. latissimus.
Foramen interventriculare (Monroi)	Porta.

With Wilder we prefer to drop the substantive *corpus* used in combination with *callosum, striatum, albicans*, etc., retaining it only when it plays the part of a useful adjective as in *corpus fornicis, corpus epididymidis*. To drop the noun and employ the adjective substantively, as in *pia* mater, membrana *mucosa*, tunica *serosa*, substantia *cinerea*, is no innovation, for in the article "Cerebrum" in James' Dictionary, published in 1743, we find *pia* used alone. If we are to employ the dionym *dura mater* we cannot consistently use the term *cavum subdurale* recommended by the "BNA."

How burdensome the use of polyonyms has become to medical men and others engaged in anatomic study is shown by the general use of *Cecum* (*cæcum* = "closed") in place of *Caput cæcum coli;* of *Tentorium* without the needless *cerebelli*, and of *Cortex* instead of *substantia corticalis* in several organs. A further potent argument in favor of mononyms, whenever these can be adopted, is suggested by the abbreviations to which they so readily lend themselves in articles and figure-legends written in different languages. Thus, as Wilder has pointed out, the dionym *commissura anterior* would be *c. a.* in the Latin and French, *a. c.* in the English, and *V. C.* in the German; but of the mononym *præcommissura* the abbreviation *prc.* would probably serve in either case and render its recognition by foreign readers easy. Not only do such mononyms lend themselves to more uniform abbreviation, but adjectives may be more readily derived, the names are more readily compounded, and by paronymy more easily adopted into other languages.

The two terms *crus* and *pedunculus* are used convertibly, without consistency, in many modern text-books. Thus, it is common to read that the *crura cerebri* enclose the *interpeduncular space*. As has already been said [2] it would have been better if anatomists had agreed to use *crus* only for the cerebral tract and *pedunculus* for the cerebellar, with, of course, the modifications made appropriate by the terms *prapedunculus, medipedunculus*, and *postpedunculus*. The word *pedunculus*, by the way, is not included in the list of general anatomic terms on p. 16.

While mononyms are to be preferred, no sensible anatomist would insist upon their adoption at the risk of ambiguity, and why *utriculus* should stand alone in the "BNA" list of structures of the internal ear, and be compounded with *prostaticus* in the list of structures in the male urethra is not explained. By the context we may understand which *vestibulum* (unaccompanied by any adjective) is meant under *Labyrinthus osseus* on p. 92, but on pp. 45, 50, 52, 57, and 59 we see the compounds *vestibulum oris, v. nasi, v. laryngis, v. vaginæ*, and *v. bursæ omentalis*.

Although it is claimed that the "BNA" list "consists chiefly of carefully selected old names" we find new, or at least comparatively unfamiliar names given to several carpal bones and the *torcular* is called *confluens sinuum*. The old *M. zygomaticus minor, M. levator labii superioris*, and the *M. levator labii superioris alæque nasi*, formidable enough as they were, are grouped under the general term *M. quadratus labii superioris*, with *caput zygomaticum, c. infraorbitale*, and *c. angulare*, corresponding to each of the above-mentioned facial muscles. McMurrich has shown the incorrectness of this classification from the morphologic standpoint.[3] Inasmuch as our anatomic vocabulary should be applicable to all vertebrates it were better, as Wilder has suggested, to employ *M. ectopectoralis* and *M. entopectoralis* for the two frequently named muscles whose relative proportions in most mammals are so misrepresented by the adjectives *major* and *minor*. On similar morphologic grounds the terms *Præcava* and *Postcava* are preferable to *Vena cava superior* and *Vena cava inferior*, and *Prasternum, Mesosternum*, and *Xiphisternum* are better and more generally applicable than the *Manubrium sterni, Corpus sterni*, and *Processus xiphoideus* of the "BNA." In speaking of the *cinerea* (*grisea* or gray matter) of the myelon the terms *Cornu dorsale* and *Cornu ventrale*, adopted by the various American associations mentioned above, are preferable to the "BNA"

[2] E. C. Spitzka, Science, April 9, 1881.

[3] Amer. Jour. Anat., III, 1, Proceedings, p. iii.

terms of *Columna posterior* and *Columna anterior* on account of their different relations in prone and erect animals. The same objection is to be offered against *posterior* and *anterior nerve-roots*, instead of the more exact *dorsal* and *ventral*. This disregard for the needs of comparative anatomy constitutes a serious imperfection in the work done by the German Commission.

The second rule adopted by the Commission reads that "each term shall be in Latin and be philologically correct." In referring to certain adjectives employed in the "BNA" which end in *-icalis*, a diligent search through Freund's Latin-German Lexikon, edited by Charlton T. Lewis and Charles Short (Harper's Latin Dictionary) fails to reveal any such termination and with regard to specific instances we find each such "BNA" term to be incorrectly constructed:

"BNA."	*Classic Latin.*
Annulus umbili*calis*	A. umbili*caris*.
Fovea supravesi*calis*	F. supravesi*caria* (*-ius*).
M. lumbri*calis*	M. lumbri*cosus*.
Substantia corti*calis*	S. corti*catus*.
Plexus cervi*calis*	Pl. cervi*catus*.

The word *cervical* (also *cervicale*) is a noun which is defined as a pillow or bolster, but there is no adjective so spelt.

There are few errors of omission. Among these we may suggest the bundle of "Purkinjé fibers" in the heart which received the attention of Purkinje and the younger His before the "BNA" was adopted, and which has since been more exhaustively investigated by Tawara, Retzer, and others under the name of *Atrioventricular bundle*. The insertion of *Foramina venarum minimarum (Thebesii)* under the list of structures in the right atrium of the heart only, seems to imply that these foramina are present in this chamber of the heart alone, whereas Thebesius, himself, mentions their existence in all of the heart chambers (1708). The ontogenetic term *Foramen ovale* should be included on p. 60, and, *Pronephros*, *Mesonephros*, and *Metanephros* on p. 58. Only one *Duodenal papilla* is mentioned. On p. 74, under "Sectiones Medullæ spinalis" we find no mention of the *Fasciculus marginalis*, or *Fasciculus dorso-lateralis*, discovered simultaneously by Lissauer and E. C. Spitzka (1886) and usually bearing the name of the former.

The Latin terms, "the only authorized ones," are accompanied by an English vocabulary which Professor Barker offers tentatively and as "simply explanatory." This caution is wisely proffered and is always to be borne in mind; for we read that *Uvula* is a *little cluster or bunch* (p. 16), *Axilla* is the *prominence of the shoulder* (p. 18), *Alveoli dentales* are defined as *tooth cavities* (instead of tooth sockets) (p. 25), while the term *tooth-cavity* is correctly given for *Cavum dentis* on p. 46. Professor Barker prefers *Carotid skein* as the English equivalent for *Glomus caroticum* (p. 53.) The literalness of the translation is at times carried too far, as, for example, in *Deep artery of the thigh* for the *A. profunda femoris* (p. 67), *Lozenge-shaped brain* for *Rhombencephalon* (p. 74), and *Writing pen* for the *Calamus scriptorius* (p. 75). It would have been better to repeat the terms *Clava* and *Obex* in the English column and put the definitions *Club* and *Bar* in parenthesis. The same with *Blue place* and *Black substance* as the equivalents of *Locus cæruleus* and *Substantia nigra*, the last-mentioned term being, in our opinion, not quite so good as *Intercalatum*.

We regret to see *Ammon's Horn* perpetuated (p. 80) even though it be only in the category of old terms; for the *Cornu Ammonis* (now designated by the better term *Hippocampus*) was not named after a man named Ammon. The erroneous form probably arose from a thoughtless translation through many past decades, of the vernacular *Corne d'Ammon* in the French, *Corno d'Ammone* in the Italian, and *Ammonshorn* in the German. We also notice the repetition of the old term *Steno's duct* (p. 46). If the term *Parotid duct* is to be at all accompanied by the name of its discoverer it should be correctly given, for it was Nickolas Stensen whose name is associated with this structure in the latinized genitive *Stenosis*.

Professor Barker shares the feeling of the members of the "Anatomische Gesellschaft" that the "BNA" is after all a provisional list and one which in that capacity has accomplished much in "clearing the decks for action." The list as it is now constituted offers much for reflection, debate, and renewed revision. It still contains stumbling-blocks for students of Anatomy and it stands in need of considerable simplification and application to comparative anatomic considerations. In the list of forty terms adopted by the American associations, twenty-five are identical with those of the "BNA" while the remaining fifteen are each and all shorter and more appropriate. With so much as a nucleus, and with the spirit of word-simplification animating the present generation, we may hope to see anatomic terminology formulated upon more logical and satisfactory lines of word construction and endowed with more lasting qualities.

EDW. ANTHONY SPITZKA.

Organic and Functional Nervous Diseases. By M. ALLEN STARR, M. D. Second edition. (*New York and Philadelphia: Lea Brothers & Company, 1907.*)

The first edition of Dr. Starr's valuable text-book appeared in 1903. The present edition offers in addition to a revision of the original text, a section of seven chapters on functional diseases of the nervous system, thus making the volume larger by sixty-five pages than the preceding one.

In the first part, on organic diseases, there are but few alterations. These consist for the most part of brief additions here and there of later observations by the author or others in symptomatology and treatment. In the histologic section two new cuts appear illustrating the disposition of the neurofibrils in the ganglion cells, and the epicellular end-structures. Two plates, originally in black, showing various normal and pathologic conditions of the nerve-cell, are attractively printed in blue monochrome. In the chapter on motor localization two new brain maps have been substituted for the old ones and the common error of locating the motor centers on both sides of the fissure of Rolando has been corrected. The treatment of the pathologic histology of paresis is hardly up to date, and cytodiagnosis as a differential diagnostic procedure receives scant notice.

The supplementary section on functional diseases treats in order the spasmodic neuroses, epilepsy, paralysis agitans, tetany, neurasthenia and occupation neuroses, hysteria and migraine, and presents six new illustrations.

The book is doubtless the best of its kind which has appeared in this country.

Saint Bartholomew's Hospital Reports. Vol. XLII, 1906. (*London: Smith, Elder & Co., 1907.*)

The value of these reports has long since been well established, and it remains but to welcome each new volume. This one contains several papers of importance, a thesis on hypertrophic pulmonary osteo-arthropathy, with a complete bibliography of this rare disease, and an article on "some diseases of the blood in children associated with enlargement of the spleen," to mention but two in the desire of attracting students to search the volume for themselves, and discover other equally noteworthy articles.

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